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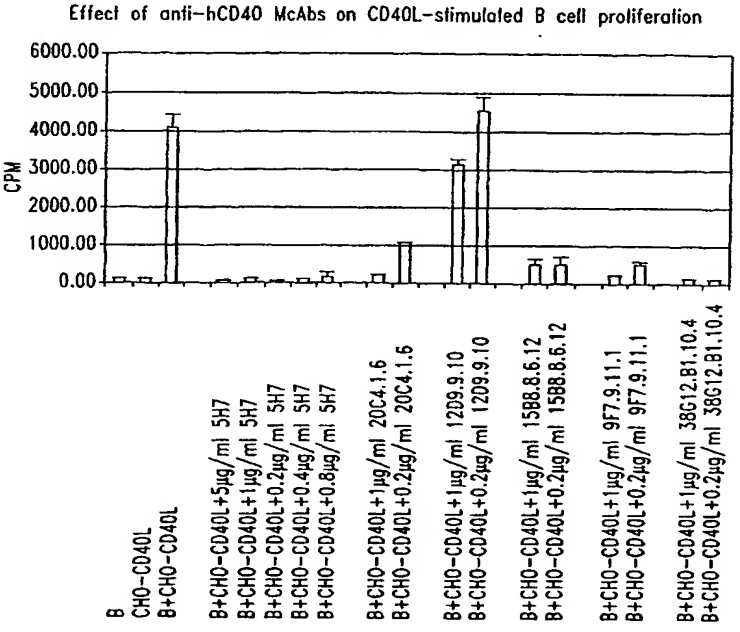
(54) Title: HUMAN ANTI-CD40 ANTIBODIES



(57) Abstract: Human antibodies capable of binding CD40 are disclosed, wherein the antibodies act as antagonists of CD40-directed activities of B cells, but have no or minimal ability to induce B cell proliferation. The antibodies are useful for treating diseases mediated by CD40-expressing cells, such as those diseases characterized by B cell activation, as well as cancer of B-cell lineage, including Non-Hodgkin's Lymphoma.

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HUMAN ANTI-CD40 ANTIBODIES

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to human antibodies capable of binding to CD40, methods of using the antibodies, and treatment of antibody-mediated disease in humans.

Description of the Related Art

The CD40 antigen is a glycoprotein expressed on the cell surface of B cells and other cells, including dendritic cells. During B-cell differentiation, the molecule is first expressed on pre-B cells and then disappears from the cell surface when the B cell becomes a plasma cell. Crosslinking of the CD40 molecules with anti-CD40 antibodies mediates a variety of effects on B cells. The CD40 antigen is known to be related to the human nerve growth factor (NGF) receptor and tumor necrosis factor-α (TNF-α) receptor, suggesting that CD40 is a receptor for a ligand with important functions in B-cell activation.

CD40 is a key element of immune responses. Engagement of CD40 on antigen-presenting cells by its ligand, termed CD40L or CD154, causes production of cytokines and up-regulation of costimulatory molecules leading to efficient activation of T lymphocytes. Engagement of CD40 on B lymphocytes provides a costimulatory signal to the B cell that drives antibody production. Thus blocking of CD40 engagement and activation has the potential to suppress antibody and cell mediation immune responses. Anti-CD40 antagonist antibodies could be used to treat autoimmune disease such as systemic lupus, psoriasis, multiple sclerosis, inflammatory bowel disease (Crohn's disease), and rheumatoid arthritis. Such antibodies could also be used to prevent rejection of organ and tissue grafts by suppressing autoimmune responses, to treat lymphomas by depriving malignant B lymphocytes of the activating signal provided by CD40, and to deliver toxins to CD40-bearing cells in a specific manner.

Previously, mouse monoclonal antibodies such as 5D12 have been disclosed that bind to CD40 without providing an activating signal. These antibodies

have the ability to inhibit immune responses *in vivo* and *in vitro*. However mouse antibodies cannot be used to treat human disease because they elicit human anti-mouse antibodies that hinder the effectiveness of the treatment. Therefore, there is a need in the art for antibodies of comparable specificity but composed of a human amino acid sequence.

BRIEF SUMMARY OF THE INVENTION

It is a primary object of this invention to provide a human monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a human B cell, wherein the binding of the antibody to the CD40 antigen prevents the growth or differentiation of the B cell.

It is a further object of this invention to provide a method for preventing or treating an antibody-mediated disease in a patient, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a human monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a CD40-bearing cell such as a human B cell or a human dendritic cell, wherein the binding of the antibody to the CD40 antigen prevents the growth or differentiation of the cell, in a pharmaceutically acceptable excipient.

It is another object of this invention to provide a method for preventing or treating an IgE-mediated disease such as an allergy in a patient, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a human monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a human B cell, wherein the binding of the antibody to the CD40 antigen prevents the growth or differentiation of the B cell, in a pharmaceutically acceptable excipient.

It is yet another object of this invention to provide a method for preventing or treating an autoimmune disease in a patient, including an antibody-mediated disease, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a human monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a human B cell, wherein the binding of the antibody to the CD40 antigen prevents the growth or differentiation

of the B cell, in a pharmaceutically acceptable excipient. Particular autoimmune diseases contemplated for treatment by this method include systematic lupus erythematosus (SLE), primary biliary cirrhosis (PBC), and idiopathic thrombocytopenic purpura (ITP).

It is another object of the invention to provide a method of inhibiting growth of tumor cells, including Non-Hodgkins Lymphoma.

In more preferred embodiments of the above objects, the monoclonal antibody is 15B8, 20C4, 13E4, 12D9, or 9F7.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 is a bar graph illustrating the effect of anti-CD40 antibodies on proliferation of B cells stimulated by CD40L-expressing cells.

Figure 2 is a bar graph illustrating the effect of anti-CD40 antibodies on B cell proliferation.

Figure 3 is a bar graph illustrating the effect of CD40 antibodies on anti-IgM-induced B cell proliferation.

Figure 4 is a bar graph illustrating the effect of crosslinked anti-CD40 antibody on proliferation of human peripheral B cells.

Figure 5 is a comparison of the amino acid sequences of the light chains of five human anti-CD40 antibodies and 5H7. The SEQ ID NOs are:

5H7: SEQ ID NOs:19, 25

9F7: SEQ ID NOs:20, 26

15B8: SEQ ID NOs:21, 27

12D9: SEQ ID NOs:22, 28

20C4: SEQ ID NOs:23, 29

13E4: SEQ ID NOs:24, 30

Figure 6 is a comparison of the amino acid sequences of the heavy chains of five human anti-CD40 antibodies and 5H7. The SEQ ID NOs are:

5H7: SEQ ID NOs:31, 37

9F7: SEQ ID NOs:32, 38

15B8: SEQ ID NOs:33, 39

12D9: SEQ ID NOs:34, 40

20C4: SEQ ID NOs:35, 41

13E4: SEQ ID NOs:36, 42

Figure 7 shows the results of FACS analysis of monoclonal antibody binding to cells expressing CD40, 15B8, showing that the antibody stained peripheral blood cells from three species: humans; Rhesus; and cynomologus macaques.

Figure 8 provides the DNA and amino acid sequences for the vK region of human monoclonal antibody 12D9, SEQ ID NOs:43 and 44, respectively.

Figure 9 provides the DNA and amino acid sequences for the heavy chain constant region of human monoclonal antibody 12D9, SEQ ID NOs:1 and 2, respectively.

Figure 10 provides the DNA sequences for the vK.1 and vh1 regions of human monoclonal antibody 20C4, SEQ ID NOs:3 and 4, respectively.

Figure 11 provides the DNA sequences for the vK.1 and vh1 regions of human monoclonal antibody 9F7, SEQ ID NOs:5 and 6, respectively.

Figure 12 provides the DNA sequences for the vK.3 and vh1 regions of human monoclonal antibody 15B8, SEQ ID NOs:7 and 8, respectively.

Figure 13 provides the DNA sequence for the vh1 regions of human monoclonal antibodies 13E4 and 12D9, SEQ ID NOs:9 and 10, respectively.

Figure 14 provides the amino acid sequences for the following regions of the indicated human monoclonal antibodies:

9F7VH1: SEQ ID NO:11

12D9VH1: SEQ ID NO:12

15B8VH1 SEQ ID NO:13

20C4VH1: SEQ ID NO:14

9F7VK1: SEQ ID NO:15

12D9VK1: SEQ ID NO:16

15B8VK1: SEQ ID NO:17

20C4VK1: SEQ ID NO:18

Figure 15 shows that anti-CD40 antibody MS81 stimulated proliferation of NHL cells in the presence and absence of IL-4, using cells from one patient.

Figure 16 shows that anti-CD40 antibody MS81 stimulated proliferation of NHL cells in the presence and absence of IL-4, using cells from a second patient.

Figure 17 shows that anti-CD40 antibody 15B8 inhibits the proliferation of NHL cells in one patient.

Figure 18 shows the dose response to 15B8 in proliferation of NHL cells from a rituxan-sensitive patient. The cells were stimulated with CD40L and IL-4.

Figure 19 shows representative dose-response curves for 15B8 effect on proliferation of human B cells stimulated by CD40L using cells from three individuals.

DETAILED DESCRIPTION OF THE INVENTION

Antibodies are constructed of several regions, a crucial region being the complementarity determining region, or CDR. The phrase "complementarity determining region" refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. *See, e.g.,* Chothia et al., *J. Mol. Biol. 196*:901-917 (1987); Kabat et al., U.S. Dept. of Health and Human Services NIH Publication No. 91-3242 (1991). The phrase "constant region" refers to the portion of the antibody molecule that confers effector functions. In previous work directed towards producing non-immunogenic antibodies for use in therapy of human disease, mouse constant regions were substituted by human constant regions. The constant regions of the subject humanized antibodies were derived from human immunoglobulins. However, these humanized antibodies still elicited an unwanted and potentially dangerous immune response in humans and there was a loss of affinity.

The human monoclonal anti-CD40 antibodies of the present invention address the shortcomings of prior art monoclonal antibodies. Accordingly, the human monoclonal antibodies of the invention are preferably produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/10741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen,

wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin-encoding loci are substituted or inactivated. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy claims, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

Using a transgenic animal described above, an immune response can be produced to a selected antigenic molecule, in this case CD40, and antibody-producing cells can be removed from the animal and used to produce hybridomas that secrete human monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein.

As used herein, the term "antibody" refers to polyclonal antibodies, monoclonal antibodies, single-chain antibodies, and fragments thereof such as Fab, $F(ab')_2$, F_v, and other fragments which retain the antigen binding function of the parent antibody.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins as well as fragments such as Fab, $F(ab')_2$, F_v, and others which retain the antigen binding function of the antibody. Monoclonal antibodies of any mammalian species can be used in this invention. In practice, however, the antibodies will typically be of rat or murine origin because of the availability of rat or murine cell lines for use in making the required hybrid cell lines or hybridomas to produce monoclonal antibodies.

As used herein, the term "single chain antibodies" refer to antibodies prepared by determining the binding domains (both heavy and light chains) of a binding antibody, and supplying a linking moiety which permits preservation of the binding function. This forms, in essence, a radically abbreviated antibody, having only that part

of the variable domain necessary for binding to the antigen. Determination and construction of single chain antibodies are described in U.S. Pat. No. 4,946,778 to Ladner et al.

The term "CD40 antigen epitope" as used herein refers to a molecule which is capable of immunoreactivity with the anti-CD40 monoclonal antibodies of this invention, excluding the CD40 antigen itself. CD40 antigen epitopes may comprise proteins, protein fragments, peptides, carbohydrates, lipids, and other molecules, but for the purposes of the present invention are most commonly proteins, short oligopeptides, oligopeptide mimics (*i.e.*, organic compounds which mimic the antibody binding properties of the CD40 antigen), or combinations thereof. Suitable oligopeptide mimics are described, *inter alia*, in PCT application US91/04282.

The antibodies of the current invention are produced by transgenic mice bearing human immunoglobulin loci, and bind to a human CD40 antigen on the surface of a human cell, particularly a B cell. These antibodies may be polyclonal antibodies, monoclonal antibodies, single-chain antibodies, and fragments thereof.

Monoclonal antibodies 15B8, 20C4, 13E4, 12D9, and 9F7 are prepared as described in the Examples. Other antibodies of the invention may be prepared similarly using mice transgenic for human immunoglobulin loci or by other methods known n the art and/or described herein.

Polyclonal sera may be prepared by conventional methods. In general, a solution containing the CD40 antigen is first used to immunize a suitable animal, in the present invention a transgenic animal, preferably a mouse bearing human immunoglobulin loci. In a preferred embodiment, Sf9 cells expressing CD40 are used as the immunogen. Immunization can also be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 μg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in

the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization.

Polyclonal antisera are obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*e.g.*, 1,000 x g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies can be prepared using the method of Kohler and Milstein, *Nature 256*:495-96 (1975), or a modification thereof. Typically, a mouse is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) are removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*e.g.*, hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the desired immunizing cell-surface antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

As an alternative to use of hybridomas, antibody can be produced in a cell line such as a CHO cell line, as disclosed in U.S. Patent Nos. 5,545,403, 5,545,405, and 5,998,144, incorporated herein by reference. Briefly, the cell line is transfected with vectors capable of expressing a light chain and a heavy chain, respectively. By transfecting the two proteins on separate vectors, chimeric antibodies can be produced. Another advantage is the correct glycosylation of the antibody.

Preferably, fully human antibodies to CD40 are obtained by immunizing transgenic mice. One such mouse is referred to as a Xenomouse, and is disclosed in U.S. Patent Nos. 6,075,181; 6,091,001; and 6,114,598, all of which are incorporated

herein by reference. To produce the antibodies disclosed herein, mice transgenic for the human IgG2 heavy chain locus and the human K light chain locus were immunized with Sf9 cells expressing human CD40. Mice can also be transgenic for other isotypes.

Production of the Sf9 (*Spodoptera frugiperda*) cells is disclosed in de Boer, U.S. Patent No. 6,004,552, incorporated herein by reference. Briefly, sequences encoding human CD40 were recombined into a baculovirus using transfer vectors as described by de Boer. The plasmids were co-transfected with wild-type baculovirus DNA into Sf9 cells. Recombinant baculovirus-infected Sf9 cells were identified and clonally purified.

Mice were injected intraperitoneally (IP) at day 0 and day 14 with 5 x 10^6 Sf9 cells expressing CD40. A final injection was made at least five weeks later, and the spleen and thymus cells were removed and used for cell fusion. Cell fusion was carried out as described by de Boer. Hybridoma antibodies were screened as described in the Examples. Five hybridomas were selected for further study, based on their ability to inhibit proliferation of human peripherial blood B cells induced by CD40 ligand (CD40L) and anti-IgM, and their ability to inhibit production of IgM by human peripheral blood B cells stimulated with anti-CD3-activated human peripheral blood T cells.

The five hybridomas showing the optimal inhibitory activity were designated 15B8.8.6 (15B8), 20C4.1.6 (20C4), 13E4.12.11 (13E4), 12D9.9.10 (12D9), 9F7.9.11.1 (9F7), and 15B8.7.2. None of these hybridomas showed significant ability to induce proliferation in resting human peripheral blood B cells.

The relative binding properties of these hybridoma antibodies was examined by flow cytometry, as described in detail in the Examples. Briefly, the antibodies compared exhibited differences in affinity despite their ability to recognize the same or closely related epitopes. For example, MAb 15B8 blocked binding of MAb 20C4 to human $CD20^+$ peripheral blood lymphocytes, but MAb 20C4 did not block MAb 15B8 binding to the $CD20^+$ lymphocytes. The differential CD40 binding of hybridomas is shown in Table 4 (Example 4).

Four hybridomas tested (15B8, 20C4, 12D9, and 9F7) produced monoclonal antibodies that stained peripheral blood cells from three species: humans; Rhesus; and cynomologus macaques (Figure 7).

To determine the polynucleotide sequences encoding the monoclonal antibodies, mRNA was prepared from the hybridomas and RT-PCR was performed on the mRNAs using standard procedures. The PCR products were analyzed on gels, sequenced, and translated. The polynucleotide and amino acid sequences are provided in SEQ ID NOs:1-18, as shown in Table 7, Example 11.

The amino acid sequences of five monoclonal antibodies (9F7, 15B8, 12D9, 20C4, and 13E4) were compared with the amino acid sequence of mouse anti-CD40 monoclonal antibody 5H7, as shown in Figures 5 (light chains) and 6 (heavy chains).

The results obtained using the five disclosed monoclonal antibodies indicate that these antibodies, as well as fragments and chimeric forms thereof, have antagonistic features that make them suitable for a number of clinical applications, including treatment of autoimmune diseases, treatment of transplantation reactions and rejections, as adjuvant therapies for gene therapies and protein therapies, and in inhibiting growth of tumor cells, including Non-Hodgkins Lymphoma cells. Additional uses include treatment of any disease mediated by a CD40-expressing malignant cell, and use to treat diseases related to the proliferation, activation, or regulation of cells expressing CD40. The activity of the five MAb's is summarized in Table 1.

Table 1

Clones	Inhibition on IgM Secretion of B cells	Inhibition on proliferation of Jurkat cell-stimulated B cells	Inhibition on proliferation of CHO-CD40L cell-stimulated B cells	Stimulation on proliferation of anti-higM-stimulated B cells	Stimulation on proliferation of B cells	Ab cross-linking to stimulate the proliferation of B cells
MS81 12D9.9.10	-29%	-64%	-24%	190%	178%	120%
MS81 15B8.8.6.12	-47%	-81%	-89%	352%	513%	76%
MS81 20C4.1.6	-35%	-74%	-97%	237%	279%	143%
MS81 13E4.12.11	-44%	-50%	n/a	346%	660%	n/a
MS81 9F7.9.11.1	-30%	-71%	-57%	120%	275%	84%

* representative data from the antibodies at 1μg/ml were shown

The invention encompasses not only the five monoclonal antibodies described herein, but also antibodies differing from these but retaining the CDR; and antibodies with one or more amino acid addition(s), deletion(s), or substitution(s), wherein the activity is measured by inhibition of B cell proliferation and/or antibody secretion. The invention also encompasses de-immunized antibodies, which can be produced as described in, for example, WO 98/52976, "Method for the Production of Non-Immunogenic Proteins," which is incorporated by reference herein. Also included within the scope of the claims are fusion proteins comprising a monoclonal antibody of the invention, or a fragment thereof, which fusion proteins can be synthesized or expressed from corresponding polynucleotide vectors, as is known in the art.

The antibodies of the present invention can have sequence variations produced using methods described in, for example, Patent Publication Nos. EP 0 983 303 A1, WO 00/34317, and WO 98/52976, incorporated herein by reference. For example, it has been shown that sequences within the CDR can cause an antibody to bind to MHC Class II and trigger an unwanted helper T cell response. A conservative substitution can allow the antibody to retain binding activity yet lose its ability to trigger an unwanted T cell response. Any such conservative or non-conservative substitutions can be made using art-recognized methods, and the resulting antibodies will fall within the scope of the invention.

The invention provides amino acid sequences for light chains and heavy chains of the preferred monoclonal antibodies (SEQ ID NOS. 1-18). The sequences are aligned as shown in Figures 5 and 6. These alignments indicate specific amino acid positions that are more amenable to substitution without loss of the desired biological activities of the antibody. Using only routine methods, one of skill in the art can construct plasmids that will encode variants of these sequences. The variant antibodies can be routinely tested for antagonistic activity, affinity, specificity, and agonistic activity using methods described herein.

An antibody produced by any of the methods described above, or any other method not disclosed herein, will fall within the scope of the invention if it possesses at least one of the following biological activities: inhibition of

immunoglobulin secretion by human peripheral B cells stimulated by T cells; inhibition of proliferation of human peripheral B cells stimulated by Jurkat T cells; and inhibition of proliferation of human peripheral B cells stimulated by CD40L-expressing cells. These assays can be performed as described in the Examples herein.

The antibodies will also exhibit a single site binding affinity (K_D) of at least 10^{-5} M, preferably at least 10^{-6} - 10^{-7} M, more preferably at least 10^{-8} M, and most preferably at least 10^{-9} M, such as 10^{-10} M, as measured using a standard assay such as Biacore, which is know in the art, in comparison with appropriate controls. Binding affinity of 10^{-11} M, 10^{-13} M, 10^{-15} M, 10^{-17} M and 10^{-19} M can also be achieved. These assays are automated, and allow the measurement of MAb specificity and cross-reactivity, which can also be assayed using standard techniques known in the art. Details of the Biacore assays are provided in Biacore's "BIAapplications handbook." Methods described in WO 01/27160 can be used to modulate the binding affinity.

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of

ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antibodies for use in the invention can be produced using any suitable technique. For example, WO 01/27160 discloses a method of conferring donor CDR binding affinity onto an antibody acceptor variable region framework. The method can also be used to optimize the binding affinity of a variable region or an antibody, such as to enhance the binding affinity. Methods for producing humanized antibodies having one or more CDR's are disclosed in U.S. Patent No. 6,180,370. Methods of producing antibodies that have been optimized for administration to humans are disclosed in WO 00/34317, which describes the production of proteins that are rendered less immunogenic or non-immunogenic. U.S. Patent No. 5,514,548 discloses methods for selection of ligand binding proteins, such as antibodies, that bind with high affinity to a target ligand. U.S. Patent No. 5,877,397 disclosed transgenic non-human animals capable of producing heterologous antibodies. All of these patents and patent publications are incorporated herein by reference.

FORMULATIONS AND METHODS OF ADMINISTRATION

The antibodies of this invention are administered at a concentration that is therapeutically effective to prevent or treat antibody-mediated diseases such as allergies, SLE, PBC, ITP, multiple sclerosis, psoriasis, Crohn's disease, graft rejection, and B cell lymphoma. To accomplish this goal, the antibodies may be formulated using a variety of acceptable excipients known in the art. Typically, the antibodies are administered by injection, either intravenously or intraperitoneally. Methods to accomplish this administration are known to those of ordinary skill in the art. It may also be possible to obtain compositions which may be topically or orally administered, or which may be capable of transmission across mucous membranes.

Before administration to patients, formulants may be added to the antibodies. A liquid formulation is preferred. For example, these formulants may include oils, polymers, vitamins, carbohydrates, amine acids, salts, buffers, albumin, surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di-, or polysaccharides, or water soluble glucans. The saccharides or

glucans can include fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. Sucrose is most preferred. "Sugar alcohol" is defined as a C_4 to C_8 hydrocarbon having an --OH group and includes galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. Mannitol is most preferred. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. Preferably, the sugar or sugar alcohol concentration is between 1.0 w/v % and 7.0 w/v %, more preferable between 2.0 and 6.0 w/v %. Preferably amino acids include levorotary (L) forms of carnitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. It is also preferred to use a buffer in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Most any physiological buffer may be used, but titrate, phosphate, succinate, and glutamate buffers or mixtures thereof are preferred. Most preferred is a citrate buffer. Preferably, the concentration is from 0.01 to 0.3 molar. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers, and methods to attach them to peptides, are shown in U.S. Pat. Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546 which are all hereby incorporated by reference in their entireties. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O--CH_2--CH_2)_n O--R$ where R can be hydrogen, or a protective group such as an alkyl or alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1000 and 40,000, more preferably between 2000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at

least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Water soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), etc. POG is preferred. One reason is because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is shown in Knauf et al., *J. Bio. Chem. 263*:15064-15070 (1988), and a discussion of POG/IL-2 conjugates is found in U.S. Pat. No. 4,766,106, both of which are hereby incorporated by reference in their entireties.

Another drug delivery system for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in Gabizon et al., *Cancer Research 42*:4734 (1982); Cafiso, *Biochem Biophys Acta 649*:129 (1981); and Szoka, *Ann Rev Biophys Eng 9*:467 (1980). Other drug delivery systems are known in the art and are described in, *e.g.*, Poznansky et al., Drug Delivery Systems (R. L. Juliano, ed., Oxford, N.Y. 1980), pp. 253-315; M. L. Poznansky, *Pharm Revs 36*:277 (1984).

After the liquid pharmaceutical composition is prepared, it is preferably lyophilized to prevent degradation and to preserve sterility. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile dilutent (Ringer's solution, distilled water, or sterile saline, for example) which may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

As stated above, the antibodies and compositions of this invention are used to treat human patients to prevent or treat antibody-mediated diseases such as allergies, SLE, PBC and ITP. The preferred route of administration is parenterally. In

parenteral administration, the compositions of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic and nontherapeutic. Examples of such vehicles are saline, Ringer's solution, dextrose solution, and Hanks' solution. Nonaqueous vehicles such as fixed oils and ethyloleate may also be used. A preferred vehicle is 5% dextrose in saline. The vehicle may contain minor mounts of additives such as substances that enhance isotonicity and chemical stability, including buffers and preservatives.

The dosage and mode of administration will depend on the individual. Generally, the compositions are administered so that antibodies are given at a dose between 1 μg/kg and 20 mg/kg, more preferably between 20 μg/kg and I 0 mg/kg, most preferably between 1 and 7 mg/kg. Suitably, it is given as an infusion or as a bolus dose, to increase circulating levels by 10-20 fold and for 4-6 hours after the bolus dose. Continuous infusion may also be used after the bolus dose. If so, the antibodies may be infused at a dose between 5 and 20 μg/kg/minute, more preferably between 7 and 15 μg/kg/minute. Suitable treatment regimens are disclosed in WO 00/27428 and WO 00/27433, which are incorporated herein by reference.

Use of Anti-CD40 Antibody Therapy for Non-Hodgkins Lymphoma Therapy

Non-Hodgkin's Lymphoma (NHL) originates from components of the spleen, thymus and lymph nodes (Jandl J.H., Non-Hogkin's lymphomas, in Jandl JH (ed): *Blood, Textbook of Hematology*, Boston, MA, Little Brown, 1996, pp. 853-887). It consists of a group of lymphocytic malignancies that are derived primarily from B and T cells. Patients with low grade NHL are usually non-responsive to radiation therapy and chemotherapy. This low response rate and the high probability of relapse contribute to the median patient survival time of fewer than 9 years.

The imbalance of growth/survival signal by CD40 and crippled death signal by Fas plays an important role in the pathogenesis of low grade B lineage malignancies, including chronic lymphcytic leukemia (CLL) and NHL (Ghia P., *Adv. Cancer Res.*, 2000, *79*:157-73). Studies in low grade NHL suggests the disease onset is due to the accumulation of the lymphoma cells as a results of reduction in apoptosis

through the Fas pathway and increase in the survival signal through CD40 (Ghia P., *Adv. Cancer Res.*, 2000, *79*:157-73). This may explain the insensitivity of the lymphoma cells to chemo or radiation therapy, which specifically target actively proliferating cells.

The invention further relates to a new NHL therapy comprising the use of an antibody to CD40 to block the survival signal for the NHL cells. This strategy is supported by a number of observations in the published scientific literature. CD40 is expressed on the surface of B cells throughout B-cell development. Studies have demonstrated that CD40 provides a survival signal for malignant B cells and stimulates their growth *in vitro* (Romano M.F. et al., *Leuk. Lymphoma*, 2000 Jan., *36*(3-4):255-62; Furman R.R., *J. Immunol.*, 2000 Feb. 15, *164*(4):2200-6; Kitada S., *Br. J. Haematol.*, 1999 Sep., *106*(4):995-1004; Romano M.F., *Blood*, 1998 Aug. 1, *92*(3):990-5; Jacob A., *Leuk. Res.*, 1998 Apr., *22*(4):379-82; Wang D., *Br. J. Haematol.*, 1997 May, *97*(2):409-17; Planken E.V., *Leukemia*, 1996 Mar., *10*(3):488-93; Greiner A., *Am. J. Pathol.*, 1997 May, *150*(5):1583-93). There is evidence from patients that the microenvironment exists to provide CD40L for CD40 signaling in vivo: CD40 is expressed on lymphoma cells in 86% of patients with B-lineage NHL (Uckun F.M., *Blood*, 1990 Dec. 15, *76*(12):2449-56). The discovery of CD40/CD40L co-expression in the same B-cell lymphoma cells raises the possibility of an autocrine growth signal loop in NHL patients (Clodi K., *Br. J. Haematol.*, 1998 Oct., *103*(1):270-5). There is also a significant increase in soluble CD40L in the NHL patient serum (Younes A., *Br. J. Haematol.*, 1998 Jan., *100*(1):135-41). The soluble CD40L can induce proliferation of lymphoma cells in primary NHL lymphoma cell culture (Andersen N.S., *Blood*, 2000 Sep. 15, *96*(6):2219-25; Buske C., *Leukemia*, 1997 Nov., *11*(11):1862-7). CD40L expression is increased in the tumor marginal zone in low-grade MALT lymphomas (Carbone A., *Am. J. Pathol.*, 1995 Oct., *147*(4):912-22; Greiner A., *Dev. Immunol.*, 1998, *6*(3-4):187-95). Given the high level of CD40 expressed on tumors of B-cell lineage and its function as a survival signal for these malignant cells, an antagonist anti-CD40 antibody may have therapeutic value in NHL.

15B8, a human IgG2 subtype anti-human CD40 monoclonal antibody generated by immunization of transgenic mice bearing the human IgG2 heavy chain

locus and the human K light chain locus (Xenomouse, Abgenix) was used. To demonstrate the potential efficacy of 15B8 in a preclinical *in vitro* model of NHL, 15B8 was tested using malignant B cells (NHL cells) obtained from NHL patients who were either rituximab treated or naïve. Rituximab is an anti-CD20 monoclonal antibody for the treatment of relapsed or refractory low grade or follicular NHL.

Since primary lymphoma cells do not proliferate in regular culture medium and undergo apoptosis after a few days in culture, tumor cells were co-cultured with irradiated CD40-ligand (CD40L) transfected feeder cells (Arpin, C., *Science*, 1995, *268*:720-722) in the presence or absence of the B cell growth factor Interleukin-4 (IL-4). Antibodies (agonist anti-CD40 MS81, or antagonist anti-CD40 15B8 or isotype control huIgG2) of indicated concentration (from 0.01 μg/ml to 10 μg/ml) were then added to the culture. Following incubation at 37°C for 48 hours, cultured cells were pulsed with ^{3}H-thymidine for 18 hours. The cells were then harvested and analyzed for the amount of ^{3}H-thymidine incorporation (Schultz, J.L., *Proc. Natl. Acad. Sci. USA*, 1995, *92*:8200-8204). All sample conditions were in triplicate.

In these NHL cell primary culture assays 15B8 alone or in combination with IL-4 did not stimulate NHL cells to proliferate *in vitro*. In contrast, an agonist anti-CD40 MS81 induced NHL cell proliferation under the same condition. 15B8 showed statistically significant inhibition of NHL cell proliferation stimulated by CD40L ($P=0.05$) and by CD40L plus IL-4 ($P<0.05$) *in vitro*. At 1-10 μg/ml or 0.1-10 μg/ml concentration range respectively, 15B8 showed a statistically significant dose related inhibition of NHL cell proliferation stimulated by CD40L or by CD40L plus IL-4 ($P<0.005$).

There are two types of preclinical models that are currently used for evaluation of human antigen-specific Mabs in therapeutic development for lymphomas. One model is the xenograft mouse *in vivo* model, where the EBV-transformed lymphoma cell lines, such as Daudi (Burkitt lymphoma) or Raji (Burkitt lymphoma) cells, are xenografted into SCID/Nude mice. However, in these models, the results only reflect effects on the particular immortal cell line, which is derived from one EBV-transformed cell. It is known that Burkitt lymphoma cells are lymphoblastoid cells (Ambinder R.F., *Cancer Treat. Res.*, 1999, *99*:27-45; Quintanilla-Martinez L., *Leuk.*

Lymphoma, 1998 Jun., *30*(1-2):111-21; Klein G., *Acta Microbiol. Immunol. Hung.*, 1996, *43*(2-3):97-105) while the lymphoma cells from NHL patients are believed to be at the mature B cell stage (Ghia P., *Adv. Cancer Res.*, 2000, *79*:157-73). EBV transformation of B cells results in changes of many components in the CD40 signaling pathway (Uchida J., *Science*, 1999 Oct. 8, *286*(5438):300-3; Farrell P.J., *Biomed. Pharmacother.*, 1997, *51*(6-7):258-67). In contrast to CD40 signaling in NHL cells and normal B cells, CD40 signaling leads to growth arrest in EBV-transformed Burkitt lymphoma cell lines (Fukuda M., *Viral Immunol.*, 2000, *13*(2):215-29; Baker M.P., *Blood*, 1998 Oct. 15, *92*(8):2830-43). Thus, the results of testing an antagonist anti-CD40 MAb (15B8) in the xenograft models will not be able to predict the response to the antibody (15B8) by NHL patients.

The other model is the *in vitro* growth inhibition assay of lymphomas cells from NHL patients, which was used herein. The advantage is that the results predicate the sensitivity of the lymphoma cells from NHL patients to the agent (15B8) tested. However, the results are obtained from *in vitro* study under defined conditions. A previously published study reported that a rat anti-mouse CD40, which failed to induce ADCC and CDD *in vitro*, showed good efficacy in two syngeneic mouse B lymphoma models (BCL1 and A31) (Tutt A.L., *J. Immunol.*, 1998 Sep. 15, *161*(6):3176-85). The anti-tumor effect on the anti-mouse CD40 occurred slower in time than an anti-Id tested. The anti-mouse CD40 may operate by blocking critical growth signals that are dependent on the expression of surface CD40 not direct signaling like anti-Id in the mouse models tested. This study suggests that the blocking of CD40/CD40L signaling by an anti-CD40 could be efficacious *in vivo*. When tested, 15B8 did not bind to the Fcγ receptors *in vitro* and failed to induce ADCC and CDC *in vitro* since it is of human IgG2 subtype. 15B8 is of similar property to the rat anti-mouse CD40. This data supports the hypothesis that 15B8 will be beneficial to NHL patients, especially Rituxan-resistant patients.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

GENERAL METHODS

ELISA Assay for Immunoglobulin Quantification

The concentrations of human IgM and IgG were estimated by ELISA. 96-well ELISA plates were coated with 2 μg/ml goat anti-human IgG MAb (The Jackson Laboratory, Bar Harbor, Maine) or with 2 μg/ml goat anti-human IgM MAb 4102 (BioSource International, Calif.) in 0.05M carbonate buffer (pH 9.6), by incubation for 16 hours at 4°C. Plates were washed 3 times with PBS-0.05% Tween-20 (PBS-Tween) and saturated with BSA for 1 hour. After 2 washes the plates were incubated for 2 hour at 37°C with different dilutions of the test samples. After 3 washes, bound Ig was detected by incubation for 2 hour at 37°C with 1 μg/ml peroxidase-labeled goat anti-human IgG MAb or goat anti-human IgM MAb. Plates were washed 4 times and bound peroxidase activity was revealed by the addition of O-phenylenediamine as a substrate. Human IgG or IgM standards (Caltaq, Burlingame, CA) was used to establish a standard curve for each assay.

Isolation of the Peripheral Blood Mononuclear Cells (PBMC) from Human Peripheral Blood.

20ml of Ficoll-Paque solution (low endotoxin, Pharmacia) was added per 50 ml polystyrene tube, in 3 tubes, 30 minutes before adding the blood. The Ficoll-Paque solution was warmed up to room temperature. 3L of bleach in 1:10 dilution was prepared, and used to wash all the tubes and pipettes contacting the blood. The blood was layered on the top of the Ficoll-Paque solution without disturbing the Ficoll layer, at 1.5ml blood/1ml of Ficoll-Paque. The tubes were centrifuged at 1700 rpm for 30 minutes at room temperature with the brake on the centrifuge turned off. As much of the top layer (plasma) as possible was removed, minimizing the vacuum in order to avoid removing the second layer of solution. The second layer, which contains the B and T lymphocytes, was collected using a sterile Pasteur pipette, and place in two 50 ml polystyrene tubes. The collection was diluted with 3x the volume of cold RPMI with no additives, and the tubes were centrifuged at 1000 RPM for 10 minutes. The media was removed by aspiratation, and the cells from both 50 ml tubes were resuspended in a

total of 10ml cold RPMI (with additives) and transferred to a 15ml tube. The cells were counted using the hemacytometer, then centrifuged at 1000 RPM for 10 minutes. The media was removed and the cells resuspended in 4mls RPMI. This fraction contained the PBMC.

Isolation of the B cells from PBMC.

100µl of Dynabeads (anti-hCD19) were placed in a 5 ml plastic tube. 3ml of sterile PBS were added to the beads and mixed, and placed in the magnetic holder, then allowed to sit for 2 minutes. The solution was removed using a Pasteur pipette. 3mls of sterile PBS were added, mixed and placed in the magnetic holder, then let sit for 2 minutes. This procedure with sterile PBS was repeated one more time for a total of 3 washes. The PBMC was added into the beads and incubated, while mixing, for 30 minutes at 4°C. The tube containing the PBMC and beads was placed into the magnetic holder for 2 minutes, then the solution was transferred to a new 5ml tube in the magnetic holder. After 2 minutes, the solution was transferred to a new 15ml tube. This step was repeated four more times, and the solutions of the first four times were collected in the 15ml tube, then centrifuged at 1000 RPM for 5 minutes. This step produced the pellet for T cell separation.

100µl RPMI (with additives) was added to collect the beads, and the solution was transferred into a 0.7ml tube. 10µl of Dynal DetachaBeads were added into the suspension at room temperature, and it was allowed to rotate for 45 minutes. The suspension was transferred into a new 5ml tube and 3mls of RPMI (with additives) was added. The tube was placed in the magnetic holder for 2 minutes. The solution was transferred into a new 5ml tube in the holder for 2 minutes, then to a 15ml tube. The previous step was repeated three more times, collecting the solution in the 15ml tube. The 15ml tube was centrifuged at 1000RPM for 10 minutes, and the cells resuspended in 10ml RMPI. The washing step was repeated 2 more times for a total of 3 washes. The cells were counted before the last centrifugation. This step completed the B cell purification. Cells were stored in 90% FCS and 10% DMSO and frozen at -80°C.

Isolation of the T Cells.

The human T cell Enrichment Column (R&D systems, anti-hCD3 column kit) was prepared using 20ml of 1X column wash buffer by mixing 2ml of 10X column wash buffer and 18ml of sterile distilled water. The column was cleaned with 70% ethanol and placed on top of a 15ml tube. The top cap of the column was removed first to avoid drawing air into the bottom of the column. Next, the bottom cap was removed, and the tip was cleaned with 70% ethanol. The fluid within the column was allowed to drain into the 15ml tube. A new sterile 15ml tube was placed under the column after the column buffer had drained to the level of the white filter. The B cell depleted PBMC fraction was suspended in 1ml of buffer and added it to the top of the column. The cells were allowed to incubate with the column at room temperature for 10 minutes. The T cells were eluted from the column with 4 aliquots of 2ml each of 1X column wash buffer. The collected T cells were centrifuged at 1000 RPM for 5 minutes. The supernatant was removed and the cells resuspended in 10mls RPMI. Cells were counted and centrifuged one more time. The supernatant was removed, completing the T cell purification. Cells were stored in 90% FCS and 10% DMSO and frozen at -80°C.

For the above procedures, the RPMI composition contained 10 % FCS (inactivated at 56°C for 45 min.), 1% Pen/Strep (100u/ml Penicillin, 0.1µg/ml Streptomycin), 1% Glutamate, 1% sodium puravate, 50µM 2-ME.

Flow Cytofluorometric Assay

Ramos cells (10^6 cells/sample) were incubated in 100 µl primary antibody (10 µg/ml in PBS-BSA) for 20 min at 4°C. After 3 washes with PBS-BSA or HBSS-BSA, the cells were incubated in 100 µl FITC-labeled $F(ab')_2$ fragments of goat anti-(human IgG) antibodies (Caltaq) for 20 min at 4°C. After 3 washes with PBS-BSA and 1 wash with PBS, the cells were resuspended in 0.5 ml PBS. Analyses were performed with a FACSCAN V (Becton Dickinson, San Jose, Calif.).

Generation of Hybridoma Clones

Splenocytes from immunized mice were fused with SP2/0 or P3 x 63Ag8.653 murine myeloma cells at a ratio of 10:1 using 50% polyethylene glycol as previously described by de Boer et al., *J. Immunol. Meth. 113*:143 (1988). The fused cells were resuspended in complete IMDM medium supplemented with hypoxanthine (0.1 mM), aminopterin (0.01 mM), thymidine (0.016 mM) and 0.5 ng/ml hIL-6 (Genzyme, Cambridge, Mass.). The fused cells were then distributed between the wells of 96-well tissue culture plates, so that each well contained 1 growing hybridoma on average.

After 10-14 days the supernatants of the hybridoma populations were screened for specific antibody production. For the screening of specific antibody production by the hybridoma clones, the supernatants from each well were pooled and tested for anti-CD40 activity specificity by ELISA first. The positives were then used for fluorescent cell staining of EBV-transformed B cells as described for the FACS Assay above. Positive hybridoma cells were cloned twice by limiting dilution in IMDM/FBS containing 0.5 ng/ml hIL-6.

EXAMPLE 1

EXPRESSION OF HUMAN CD40 IN SF9 CELLS

Sf9 insect cells infected with recombinant virus Autographa californica baculovirus (AcNPV), encoding CD40, were cultured for 48 hours in 24-well plates. After removal of the tissue culture medium the plates were incubated for 45 minutes at room temperature (RT) with 0.25 ml of antibody in PBS with 1% BSA (PBS-BSA). After three washes with PBS-BSA, the plates were incubated for 35 minutes at RT with 250 µl of a 1/250 dilution of goat anti-(mouse total Ig) immunoglobulins conjugated to horseradish peroxidase (Zymed, South San Francisco, Calif.) in PBS-BSA. Unbound peroxidase activity was removed by washing five times with PBS-BSA. Bound peroxidase activity was revealed by the addition of an assay mixture prepared by diluting 0.5 ml of 2 mg/ml 3,3',5,5'-tetramethylbenzidine in ethanol to 10 ml with 10 mM sodium acetate, 10 mM EDTA buffer (pH 5.0) and adding 0.03% (v/v) H_2O_2. The reaction was stopped after 10 minutes by adding 100 µl of 1 M H_2SO_4.

EXAMPLE 2

ANTIBODIES RAISED IN HUMAN IMMUNOGLOBULIN TRANSGENIC MICE

Mice transgenic for the human IgG2 heavy chain locus and the human K light chain locus were immunized with Sf9 cells expressing human CD40. The method of immunization was carried out as described by de Boer, U.S. Patent No. 6,004,552. Briefly, mice were injected intraperitoneally at day 0 and day 14 with 5×10^6 Sf9 cells infected with AcCD40 virus. At day 21, 100 µl of serum was obtained to test for the presence of specific antibodies. After a rest period of at least two weeks, the mice received a final injection with 5×10^6 cells infected with AcCD40 virus. Three days after this last injection, the spleen cells were used for cell fusion.

Mice were selected for fusion based on the reactivity of their sera with recombinant CD40 in an ELISA. Spleen cells from immunized mice were fused with mouse myeloma cells (NS/0) by the method of Kohler and Milstein, *Nature 256*:495-96 (1975), with modifications. Hybridomas grown in HAT selective medium were selected for further characterization based on their ability to bind CD40 in an ELISA. Hybridomas that produced antibodies that bound nontransfected Sf9 cell lysate or anti-mouse light chain antibody were dropped from consideration. Hybridomas that produced CD40-binding antibodies that did not bind Sf9 cell lysate or anti-mouse light chain antibody were subcloned. Subclones were used to produce antibodies for further characterization. Hybridoma antibodies were also tested for their ability to stain Ramos lymphoma cells, which express human CD40 on their surfaces. The concentration of antibodies giving half-maximal CD40 binding as measured by ELISA is shown below in Table 2.

Table 2

Concentration of anti-CD40 antibody giving half-maximal CD40 binding by ELISA

Antibody	EC50 (ng/ml)
5H7	10
12D9	15
15B8	40
20C4	15
9F7	15

Hybridoma antibodies were then selected for their ability to inhibit the production of IgM by human peripheral blood B cells stimulated with anti-CD28-activated human peripheral blood T cells (Figure 1). Hybridoma antibodies were further screened for their ability to inhibit proliferation of human peripheral blood B cells induced by CD40L and anti-IgM (Figure 2). Hybridomas were also screened for their ability to induce proliferation in resting human peripheral blood B cells (Figure 3). Some hybridomas, such as 36C4-G2, exhibited marked stimulatory activity. Thus, even when able to bind CD40, not all human antibodies exhibit the desired inhibitory effect. Four hybridomas were selected from approximately 36 as having the optimal inhibitory activity.

EXAMPLE 3

BINDING PROPERTIES OF SELECTED HYBRIDOMAS

Four hybridomas were selected based on their inhibitory effect on B cell activation as described above in Example 2. Their binding properties were determined by BIAcore evaluation using soluble recombinant CD40 as the mobile phase with the anti-CD40 antibodies being captured on the sensor surface. The inhibitory antibodies exhibited various binding affinities, which are suitable for the uses described herein, as shown in Table 3.

Table 3

Antibody	Ka (M-1s-1)	Kd (sec^{-1})	KD (M)
5H7 F_{ab}*	2.4×10^6	3.5×10^{-3}	1.4×10^{-9}
5H7 Ab**	1.5×10^6	4.1×10^{-3}	2.8×10^{-9}
15B8**	1.16×10^6	3.6×10^{-3}	3.1×10^{-9}
20C4**	1.9×10^5	3.6×10^{-2}	1.8×10^{-7}
12D9**	1.5×10^5	1.0×10^{-1}	6.7×10^{-7}
9F7**	1.35×10^5	2.2×10^{-2}	1.7×10^{-7}

*Fixed phase = CD40, Mobile phase = F_{ab}

**Fixed phase = antibody, Mobile phase = sCD40

EXAMPLE 4

VARIABLE HYBRIDOMA BINDING TO CD40

The relative binding properties of the selected monoclonal antibodies were examined by flow cytometry. Whole blood was incubated for 30 minutes with various concentrations of unlabeled hybridoma antibody plus 0.1 µg of FITC-conjugated 15B8 and anti-CD20-PECy5. The RBC's were then lysed, the leukocyte populations fixed, and the preparations acquired for analysis.

These studies show that unlabeled 15B8 blocks the binding of unlabeled 20C4 to CD20+ lymphocytes from human peripheral blood (Table 4). In contrast, unlabeled 20C4 only weakly blocked the binding of labeled 15B8 to the same cell population. Thus, although the two antibodies recognize the same or closely associated epitopes, the difference in affinity shown in the Biacore analysis also is reflected in the weaker competitive ability of the lower-affinity antibody. The labeled MAb's were used to stain peripheral blood cells from humans, Rhesus (*Macaca mulatta*), and cynomolgus (*Macaca fascicularis*) macaques. All three species were stained by the 15B8, 20C4, 12D9, and 9F7 MAb's (Figure 7).

Table 4

Tube #	Antibody-1	µg	Antibody-2	µg	huWB #1219	MFI*	% MFI reduction
1	-	-	15B6-FITC	0.1	100µl	72.0	
2	HulgG2	0.1	15B8-FITC	0.1	100 µl	117.5	-63.19%
3	HulgG2	0.5	15B8-FITC	0.1	100 µl	97.0	-34.72%
4	HulgG2	1	15B8-FITC	0.1	100 µl	103.8	-44.17%
5	15B8	0.1	15B8-FITC	0.1	100 µl	22.0	69.44%
6	15B8	0.5	15B8-FITC	0.1	100 µl	20.5	71.53%
7	15B8	1	15B8-FITC	0.1	100 µl	25.9	64.03
8	20C4	0.1	15B8-FITC	0.1	100 µl	91.6	-27.22%
9	20C4	0.5	15B8-FITC	0.1	100 µl	70.3	2.36%
10	20C4	1	15B8-FITC	0.1	100 µl	61.0	15.28%
11	-	-	20C4-FITC	0.1	100 µl	82.3	
12	HulgG2	0.1	20C4-FITC	0.1	100 µl	82.6	-0.61%
13	HulgG2	0.5	20C4-FITC	0.1	100 µl	74.4	9.60%
14	HulgG2	1	20C4-FITC	0.1	100 µl	82.4	-0.12%
15	15B8	0.1	20C4-FITC	0.1	100 µl	18.3	77.76%
16	15B8	0.5	20C4-FITC	0.1	100 µl	21.3	74.12%
17	15B8	1	20C4-FITC	0.1	100 µl	22.3	72.90%
18	20C4	0.1	20C4-FITC	0.1	100 µl	30.2	63.30%
19	20C4	0.5	20C4-FITC	0.1	100 µl	22.6	72.54%
20	20C4	1	20C4-FITC	0.1	100 µl	20.8	74.73%

* MFI: mean fluorescent intensity

EXAMPLE 5

INHIBITION OF IMMUNOGLOBULIN SECRETION BY HUMAN PERIPHERAL B CELLS

Plates were coated with anti-human CD3 (2µg/ml, UCHTi, NE/LE, Pharmingen), at 4°C overnight. The pre-coated plates were washed 3 x with PBS. The T cells were irradiated with 3000 Rad. The B cells were resuspended in RPMI(+) to 10^4 per ml. 100µl of B cells were added into the well, then anti-CD40 antibodies were added into the well. The T cells were resuspended in RPMI(+) to 10^5 per ml. Human recombinant IL2 to 200u/ml was added (Chiron, 10u/µl water solution stored at –20°C) was added in the cell suspension. A 100µl suspension was taken into each well, and mixed well with the B cells and antibodies. RPMI(+) was added to the wells to a total of 200µl. The plates were incubated at 37°C for 8 days before harvesting the media, after spinning down the cells for ELISA. The results are shown below in Table 5.

Table 5

Effect of monoclonal antibodies on IgM secretion by T cell-stimulated B cells

Antibody	O.D.
None	0.67
15B8	0.35
20C4	0.43
12D9	0.47
13E4	0.37

The results show that in the presence of an antibody according to the invention, the secretion of immunoglobulin, IgM, by T cell-stimulated human peripheral blood B cells, is decreased.

EXAMPLE 6

INHIBITION OF PROLIFERATION OF JURKAT-STIMULATED HUMAN PERIPHERAL B CELLS

B cells were purified as described above. 10^4 purified B cells, 10^5 irradiated Jurkat cells (3000 Rad), and antibodies to be tested were added into anti-CD3-coated 96-well plates. The plates were incubated at 37°C for four days, with labeling of the cells with ^{3}H-thymidine during the last 18 hours. The cells were harvested and counted. The results are shown below in Table 6.

Table 6

Effect of antibodies on Jurkat cell-stimulated B cell proliferation

Antibody	Counts per minute (cpm)
None	14,000
5H7, 10 μg/ml	8,000
5H7, 0.1 μg/ml	9,000
15B8, 1 μg/ml	10,500
13E4, 1 μg/ml	11,000
20C4, 1 μg/ml	10,500

The results indicate that in the presence of the antibodies of the invention, B cell proliferation stimulated by Jurkat cells, is inhibited.

EXAMPLE 7

INHIBITION OF PROLIFERATION OF CD40L-STIMULATED HUMAN PERIPHERAL B CELLS

B cells were purified as described above. 10^4 purified B cells, 2 x 10^4 Formaldehyde-fixed CHO-CD40L cells, and antibodies to be tested were added into anti-CD3-coated 96-well plates. The plates were incubated at 37°C for four days, with labeling of the cells with ^{3}H-thymidine during the last 18 hours. The cells were harvested and counted. The results are shown below in Figure 1.

EXAMPLE 8

STIMULATION OF B CELL PROLIFERATION

B cells (1 x 10^4 per well) were cultured in 200 µl RPMI supplemented with 10% fetal calf serum in U-bottom 96-well microtiter plates. B cells were stimulated by addition of immobilized anti-(IgM) antibodies (5 µg/ml, Sigma). Varying concentrations of MAbs were added at the onset of the microcultures and proliferation was assessed at day 4 by measurement of the incorporation of ^{3}H-thymidine after 18 hour pulsing. The results are shown in Figure 2.

EXAMPLE 9

EFFECT OF ANTI-CD40 ANTIBODIES ON PROLIFERATION OF HUMAN PERIPHERAL B CELLS STIMULATED BY ANTI-IGM

10^4 purified B cells, anti-IgM beads (5 µg/ml, Sigma), and antibodies to be tested were added into 96 well plates. The plates were incubated at 37°C for four days, with labeling of the cells with ^{3}H-thymidine during the last 18 hours. The cells were harvested and counted, and the results are shown in Figure 3.

EXAMPLE 10

EFFECT OF ANTI-CD40 ANTIBODY CROSSLINKING ON PROLIFERATION OF HUMAN PERIPHERAL B CELLS

10^4 purified B cells were added into anti-CD40-coated 96 well plates. The plates were incubated at 37°C for four days, with labeling of the cells with ^{3}H-

thymidine during the last 18 hours. The cells were harvested and counted, and the results are shown in Figure 4.

EXAMPLE 11

POLYNUCLEOTIDE AND AMINO ACID SEQUENCES OF HUMAN ANTI-CD40 ANTIBODIES

mRNA was prepared from the hybridomas generated as described in Example 2 and RT-PCR was performed on the mRNAs. Two sets of primers were used to generate PCR products: a universal or pool of heavy and light chain family primers; and then family-specific primers. The PCR products were analyzed on gels, sequenced, and translated. The polynucleotide and amino acid sequences are provided in the Sequence Listing as summarized in Table 7.

Table 7

SEQ ID NO:	Antibody	Region
1	12D9	Heavy chain constant region polynucleotide
2	12D9	Heavy chain constant region amino acids
3	20C4	VK1 polynucleotide
4	20C4	VH1 polynucleotide
5	9F7	VK1 polynucleotide
6	9F7	VH1 polynucleotide
7	15B8	VK3 polynucleotide
8	15B8	VH1 polynucleotide
9	13E4	VH1 polynucleotide
10	12D9	VH1 polynucleotide
11	9F7	VH1 amino acids
12	12D9	VH1 amino acids
13	15B8	VH1 amino acids
14	20C4	VH1 amino acids
15	9F7	VK1 amino acids
16	12D9	VK1 amino acids
17	15B8	VK1 amino acids
18	20C4	VK1 amino acids

Specific regions of the antibodies are shown in Figures 5, 6, and 8-14. This information can be used to design additional monoclonal antibodies for use according to the invention. These monoclonal antibodies may differ from those described herein, by substitution of one or more of the framework or CDR regions. The monoclonal antibodies also may differ by substitution of one or more amino acids, which are shown to differ in certain regions of the framework and CDR (Figures 5 and 6). Once the amino acid sequence is designed, routine procedures can be used to construct a corresponding polynucleotide sequence for expression of the monoclonal antibody. Expression and purification of the monoclonal antibodies is performed using methods known in the art, such as those disclosed in U. S. Patent Nos. 5,545,403, 5,545,405, and 5,998,144, which are incorporated herein by reference.

EXAMPLE 12

EFFECT OF 15B8 ON MALIGNANT B-CELL PROLIFERATION IN *IN VITRO*

To test if 15B8 provides the growth signal like CD40L *in vitro*, B cells from tumor infiltrated lymph nodes (NHL cells) were obtained from one antibody naïve, one rituximab-sensitive and one rituximab-resistant NHL patient. The NHL cells were studied under four different culture conditions; no added antibody (medium); addition of human isotype antibody IgG2 (control); addition of anti-CD40 antibody MS81 (agonistic antibody); and addition of 15B8. All antibodies were tested 1, 2, and 5 μg/mL in the presence or absence of IL-4. The NHL cells from two patients were cultured as described above under the same four conditions in the presence of IL-4 (2 ng/ml). B-cell proliferation was measured by ^{3}H-thymidine incorporation as described above.

Anti-CD40 antibody 15B8, at concentration of 1, 2 and 5 μg/mL, did no stimulate NHL cells to proliferate in either the presence or absence of IL-4. In contrast, an agonistic anti-CD40 antibody (MS81), tested at the same concentration, stimulated NHL-cell proliferation or in the presence and absence of IL-4 in all patient samples. Representative results from one patient are shown below (Fig. 15 and Fig. 16). Results from the NHL cells from the two patients in the presence of IL-4 and three patients in the absence of IL-4 were comparable. These results indicate that 15B8 is not an agonist

anti-CD40 antibody and does not stimulate proliferation of NHL cells from rituximab-sensitive, naïve or -resistant NHL patients *in vitro*.

FACS analysis of the NHL cells was performed with either a direct labelled 15B8-FITC or 15B8 plus anti-HuIgG2-FITC to confirm that CD40 is expressed on the surface the NHL cells tested and that 15B8 binds to the NHL cells. The NHL cells from two rituximab-sensitive and four rituximab-resistant patients (6 patients in total) were tested. NHL cells from all the patients expressed CD40 and bound to 15B8. The 15B8 binding-positive cell population in any given patient is about 66% to 91%.

EXAMPLE 13

15B8 INHIBITS CD40L-STIMULATED PROLIFERATION OF NHL CELLS *IN VITRO*

To evaluate the ability of 15B8 to block the growth signal provided by CD40L *in vitro*, NHL cells from patients were cultured as described in Example 12 in suspension over CD40L-expressing feeder cells under four different conditions: no added antibody (medium); addition of human isotype antibody IgG2 (control); addition of anti-CD40 antibody MS81 (agonistic antibody); and addition of 15B8. All antibodies were added at concentration of 1, 2, and 5 μg/mL in the presence or the absence of IL-4. The NHL cells from one antibody naïve, two rituximab-sensitive and five rituximab-resistant patients (8 patients in total) were cultured under the same four conditions as described above in the presence of IL-4 (2 ng/ml). NHL cells from three rituximab-sensitive and four rituximab-resistant patients (7 patients in total) were cultured under similar conditions in the absence of IL-4. The NHL cell proliferation was measured by ^{3}H-thymidine incorporation.

Table 8 below shows the inhibitory effect of 15B8 on the proliferation of NHL cells from two rituximab-sensitive (data from one patient reproducible in two separate experiments) and four rituximab-resistant patients (6 patients in total) stimulated by CD40L alone *in vitro*. Representative results from the cells of one patient (A) are shown (Fig. 17). 15B8 inhibited the proliferation by about 12-68% when compared to the control in the six patients. The degree of inhibition by 15B8 varies depending on patient samples and the dose level of 15B8. Statistical analysis of the data from six of the seven patient samples tested shows that the inhibition of CD40L-

stimulated NHL cell proliferation by 15B8 is significant at 1 μg/ml ($p=0.05$). There is a statistically significant dose response ($p<0.005$), the inhibitory effect increases with increasing 15B8 dose.

Table 8

Effect of 15B8 Mab on CD40-L Stimulated Proliferation of NHL Patient Cells in the Absence of IL-4[1]

patient ID	patient type[2]	treatment dose (ug/ml)	15B8 % inhib.[3]
A	CR	1	56.61
		2	58.99
		5	63.16
A	CR	1	61.96
		2	60.41
		5	64.75
		10	60.29
B	CR	1	none
		2	none
		5	none
		10	12.11
D	NR	1	52.22
		2	61.63
		5	68.04
		10	68.17
E	NR	1	13.07
		2	22.34
		5	31.04
		10	31.87
F	NR	1	24.51
		2	27.43
		5	38.71
		10	47.35
G	NR	1	11.12
		2	22.41
		5	30.61
		10	43.15

1. NHL cells from patients were cultured with murine L-cells expressing human CD40L in the presence of medium, agonist anti-CD40 (MS81), antagonist anti-CD40 (15B8) or huIgG2 isotype control *in vitro*. The proliferation of the NHL cells was measured by 3H-thymidine incorporation (data from one rituximab-sensitive patient is not in the table for the cpm of CD40L is<2000).

2. Patient response to anti-CD20 Mab therapy; CR, complete responder; NR, nonresponder

3. 15B8 % inhibition = 100 - (15B8 cpm/hulgG2 cpm x 100); represents the mean of 3 determinations

Table 9 (below) shows the inhibitory effect of 15B8 on proliferation of NHL cells from one antibody naïve, two rituximab-sensitive (data from both patient samples were repeated twice reproducibly) and five rituximab-resistant patients (8 patients in total) stimulated by both CD40L and IL-4 *in vitro*. At 1 μg/ml, 15B8 significantly ($p<0.05$) inhibited the CD40L and IL-4-mediated proliferation of the NHL cells. The degree of inhibition ranged from 18-69% at high dose (5 or 10 μg/ml) in samples from all 8 patients *in vitro*. There is a statistically significant dose response of this inhibitory effect by 15B8 ($p<0.005$) (Fig. 18 shows one representative dose response curve) at 15B8 concentration range of 0.01 - 10 μg/ml.

These *in vitro* results suggest that treatment with 15B8 may block the CD40-mediated growth signal for NHL cells in patients.

Table 9

Effect on 15B8 MAb on CD40-L Stimulation of NHL Patient Cells in the Presence of IL-4[1]

patient ID	patient type[2]	treatment dose (ug/ml)	15B8 % inhib.[3]
A	CR	1	34.39
		2	30.54
		5	36.42
A	CR	0.01	0.44
		0.04	23.32
		0.2	29.54
		1	35.38
		5	46.12
		10	48.63
C	CR	1	34.91
		2	40.89
		5	56.34
		10	69.21
C	CR	1	none
		2	16.79
		5	21.64
		10	12.63
D	NR	1	1.95
		2	6.43

patient ID	patient type[2]	treatment dose (ug/ml)	15B8 % inhib.[3]
		5	20.95
		10	26.31
E	NR	1	1.91
		2	2.74
		5	28.36
		10	28.26
F	NR	1	none
		2	11.76
		5	27.54
		10	34.07
G	NR	1	39.38
		2	32.74
		5	36.48
		10	37.78
H	NR	1	none
		2	none
		5	7.81
		10	18.47
I	Naïve	0.01	none
		0.04	13.16
		0.2	15.64
		1	16.20
		5	21.53
		10	24.51

1. NHL cells from patients were cultured with murine L-cells expressing human CD40L in the presence of IL-4 (human interleukin-4) at 2 ng/ml under conditions described in Table 1.
2. Patient response to anti-CD20 Mab therapy; CR, complete responder; NR, nonresponder; Naïve, untreated
3. % inhibition compared to hIgG2. 15B8 % inhibition = 100- (15B8 cpm/hulgG2 cpmx100)

EXAMPLE 14

DEMONSTRATION OF AGONISTIC AND ANATAGONISTIC ACTIVITY OF 15B8 IN DIFFERENT SPECIES *IN VITRO*

To determine if it is an agonist or antagonist anti-CD40, 15B8 was tested in several *in vitro* assays described below using cells from humans and five different primate species, including champanzee (chimp), marmoset, cynomologus mokey, rhesus monkey and baboon.

15B8 does not activate human peripheral blood B cell and does not cause PBMC proliferation *in vitro* in human, chimp and marmoset. Activation of CD40 on human B cells obtained from peripheral blood leads to proliferation of the B cells (van Kooten C., *J. Leukoc. Biol.*, 2000 Jan., *67*(1):2-17; Denton M.D., *Pediatr. Transplant.*, 1998 Feb., *2*(1):6-15; Evans D.E., *J. Immunol.*, 2000 Jan. 15, *164*(2):688-97; Noelle R.J., *Agents Actions Suppl.*, 1998, *49*:17-22; Lederman S. et al., *Curr. Opin. Hematol.*, 1996, *3*(1):77-86). To test if 15B8 activates CD40 on B cells, a series of proliferation assays was carried out using freshly isolated human B cells or PBMCs from peripheral blood. The effect of 15B8 in this assay was measured by ^{3}H methyl-thymidine incorporation (John E. Coligan et al., *Current Protocols in Immunology*, Vol. 13:12, John Wiley & Sons, Inc., 1991; Kwekkeboom J., *Immunology*, 1993 Jul, *79*(3):439-444). As shown in Table 10 below at concentrations of 0.2, 1 and 5 μg/ml, 15B8 had minimal effect on purified B cell proliferation compared to the effect on CD40L, which demonstrated strong proliferation-promoting effect on human B cells. As shown in table 3, the results in purified B cells from two healthy volunteers are comparable.

15B8 was further compared to CD40L for stimulation of human PBMC proliferation using freshly isolated human PBMC. As summarized in Table 10 below, 15B8 does not stimulate human PBMC proliferation *in vitro* as measured by 3-H methyl-thymidine incorporation (John E. Coligan et al., *Current Protocols in Immunology*, Vol. 13:12, John Wiley & Sons, Inc., 1991; Kwekkeboom J., *Immunology*, 1993 Jul, *79*(3):439-444) in samples tested from sixteen volunteers at concentration range of 0.2-5μg/ml.

Table 10

Stimulation of PBMC/B cell-proliferation in human, chimp and marmoset by 15B8 antibody[1]

Species	cell source	Number of samples	dose (ug/ml)	hIgG2, base	CD40L, fold increase[3]	15B8, fold increase[2]
human	B	2	5	1	70.58/36.33	1.77/4.37
		2	1	1	70.58/36.33	3.1/5.4
		2	0.2	1	70.58/36.33	1.16/4.63
human	PBMC	5	5	1	9.36-91.60	0.40-2.28
		15	1	1	9.36-91.60	0.35-2.38
		12	0.2	1	9.36-91.60	0.41-3.74
Marmoset Monkey	PBMC	3	5	1	29.24-90.3	2.05-7.2
		5	1	1	7.99-90.3	1.35-5.79
Chimp PBMC	PBMC	1	5	1	10.15	2.46
		5	1	1	5.12-9.2	0.66-5.2

1. B cells/PBMCs were cultured in vitro in the presence of CD40L, 15B8 or huIgG2 isotype control.
2. Results of the cell proliferation are reported as the ration of 3H-thymidine incorporation for 15B8 to huIG2 controls.
Data from some samples are not included in the table for the CPM induced by CD40L (positive control) <2000.
3. The fold-increase for CD40L shown in the table is the ratio of the CD40L cpm to the cpm of huIG2 at 5μg/ml.
CD40L: CD40L transfected CHO cells, fixed with formaldehyde before the experiments.

Upon B cell activation, a number of cell surface proteins are up-regulated (Denton M.D., *Pediatr. Transplant.*, 1998 Feb., *2*(1):6-15; Evans D.E., *J. Immunol.*, 2000 Jan. 15, *164*(2):688-97; Noelle R.J., *Agents Actions Suppl.*, 1998, *49*:17-22; Lederman S. et al., *Curr. Opin. Hematol.*, 1996, *3*(1):77-86). To confirm that 15B8 does not activate human B cells and does not induce an agonist signal when bound to CD40, its ability to up-regulate B cell activation markers was tested by FACS analysis using purified human PBMC. There was no up-regulation in the expression of activation markers such as CD25, CD69, CD86, HLA-DR and ICAM-1 (CD54) in 15B8 treated human B cells (Table 4). The level of these markers was similar when cells were treated with either 15B8 or huIgG2 control (Table 11). In contrast, CD69 was consistently up-regulated by CD40L in PBMC samples from three healthy volunteers tested.

Table 11

Effect of 15B8 on upregulation of B-cell activation markers in vitro by FACS

Species	cell source	incubation time	Number of subject	CD54	CD69	HLA-DR	CD25	CD80	CD86
human	CD20 from PBMC	4 h-24 h	3	-	-	-	-	N/A	-
Chimp	CD20 from PBMC	4h-24h	3	N/A	-	N/A	N/A	N/A	N/A

1 "-" means no up-regulation.
2 "N/A": means not measured or not successful.

Additional consequences of B cell activation are up-regulation of surface FasL and apoptosis (Revy P., *Eur. J. Immunol.*, 1998 Nov., *28*(11):3648-3654; Carey G.B., *Immunol. Rev.*, 2000 Aug., *176*:105-115; Ju S.T., *Int. Rev. Immunol.*, 1999, *18*(5-6):485-513; Baumgarth N., *Immunol. Rev.*, 2000 Aug., *176*:171-180). To confirm that 15B8 is not an agonistic anti-CD40 antibody, its ability to induce FasL expression and apoptosis of human B cells was also tested. Annexin V staining on the cell surface can be used as an early apoptosis marker (Ju S.T., *Int. Rev. Immunol.*, 1999, *18*(5-6):485-513). Human B cells were purified from peripheral blood and incubated with 15B8. FACS analysis was used to detect cells with positive staining of Annexin V and anti-FasL. There was no significant difference on the surface staining by the two reagents between cells incubated with 15B8 or the isotype control (huIgG2) antibody. This result shows that 15B8 does not induce apoptosis of human B cells *in vitro*. These data provide further evidence that 15B8 is not an agonistic anti-CD40 antibody for human B cells.

15B8 cross-reacts with CD40 expressed on the surface of CD20 positive PBMCs from primates. To test if 15B8 can activate CD40 on B cells from other primate species such as chimps and marmosets, the same proliferation assay were carried out using freshly isolated chimp and marmoset PBMC from fifteen chimps and five marmosets. Similar to the results with the human PBMC, 15B8 did not stimulate the proliferation *in vitro* of PBMCs from six chimps and five marmosets at 1 and 5 μg/ml concentration (Table 3 above). 15B8 also did not up-regulate the expression of activation marker, CD69, in the three chimp-PBMC samples tested (Table 4). 15B8 did

not show any effect on FasL expression and apoptosis in chimp PBMCs similar to human PBMC controls after 24 and 48 hours simulation in vitro in all samples from six chimps tested.

Cross-linking 15B8 by a secondary antibody fixed to plastic surface did not increase its potency to stimulate B cell proliferation (data not shown). When tested using PBMCs from humans and chimps in this cross-linking assay, 15B8 did not stimulate proliferation of the cells. This observation indicates a reduced risk of 15B8 being stimulative (agonistic) for B cell proliferation in case of induction of anti-15B8 (HAHA) or Fc binding to other Fc receptor expressing cells when administered *in vivo*.

In summary, 15B8 does not initiate an activation signal in human B cells/PBMCs nor in chimp/marmoset PBMCs *in vitro*. Therefore, 15B8 is not an agonistic anti-CD40 antibody in humans, chimps and marmosets.

EXAMPLE 15

15B8 IS AN ANTAGONIST ANTI-CD40 ANTIBODY IN HUMANS, CHIMPS AND MARMOSETS *IN VITRO*.

To determine if 15B8 is an antagonist anti-CD40, its ability to inhibit CD40-CD40L interaction was tested in a CD40L mediated-human B cell proliferation assay (Kwekkeboom J., *Immunology*, 1993 Jul, *79*(3):439-444). A transfected CHO cell line expressing human CD40L was used to stimulate the proliferation of purified human peripheral blood B cells or PBMCs. Human B cells from ten healthy volunteers and human PBMCs from three healthy volunteers were tested. In all the samples tested, 15B8 suppressed CD40L-expressing CHO cells mediated-proliferation by 42-88% at concentration range from 0.2 - 5μg/ml (Table 12). Figure 19 shows representative dose-response curves using cells from three individuals. The no-effect dose of 15B8 is 0.008 μg/ml and reaches saturating dose at 0.2 μg/ml (Figure 19). This observation indicates that 15B8, as an antagonist anti-CD40 antibody, can inhibit the growth signals in human B cells and PBMCs provided by cell surface-expressed CD40L.

Table 12

Inhibition of CD40L induced-proliferation of PBMC/B cell with 15B8 antibody[1]

Species	cell source	Number of samples	dose (ug/ml)	CD40L (base)	hIgG2, % of inhibition	15B8, fold inhibition2
human	B	7	5	100	(-27) - 14%	45-85%
		9	1	100	(-93) -11%	42-87%
		6	0.2	100	(-20) - (-6)%	44-82%
human	PBMC	1	5	100	13%	45%
		2	1	100	3-32%	76-88%
Marmoset Monkey	PBMC	3	1	100	1-35%	68-84%
Chimp PBMC	PBMC	3	1	100	(-3) - 21%	55-73%

1. B cells/PBMCs were cultured *in vitro* with CD40L expressing CHO cells in the presence of 15B8 or huIgG2 control. CD40L transfected CHO cells were fixed with formaldehyde before the experiments. The proliferation of cells was measured by 3H-thymidine incorporation.
2. "15B8 % inhibition" = 100- (15B8 cpm/CD40L cpm x 100)
Data from some sample are not in the table for proliferation induced by CD40L (positive control) is -folds.

Additional assays were carried out using freshly isolated PBMCs from nine chimps and three marmosets. As with the human PBMCs, 15B8 was able to inhibit the proliferation of chimp and marmoset PBMCs stimulated by CD40L expressing-CHO cells at 1μg/ml concentration level (Table 5 above). The inhibition by 15B8 was approximately 55-73% and 68-84% in PBMC samples from three chimps and three marmosets respectively (Table 5 above).

Activated B cells undergo a number of biological response such as proliferation and antibody production. The activation of B cells by T cell-dependent antigens involves $CD4^+$ T-helper (Th) cells. This T cell helper process is mediated by a concerted effort of the interaction of CD40 on the B cells with the CD40L on the Th cells surface together with the interactions of other co-stimulatory factors and cytokinesDenton M.D., *Pediatr. Transplant.*, 1998 Feb., *2*(1):6-15; Evans D.E., *J. Immunol.*, 2000 Jan. 15, *164*(2):688-97; Noelle R.J., *Agents Actions Suppl.*, 1998, *49*:17-22; Lederman S. et al., *Curr. Opin. Hematol.*, 1996, *3*(1):77-86; Mackey M.F., et al., *J. Leukoc. Biol.*, 1998, *63*(4):418-428). To test if 15B8 can block T-helper cell mediated B cell antibody production, purified human peripheral blood B cells were cultured in the presence of purified irradiate T cells activated with anti-CD3 antibody. An ELISA assay was used to measure the level of IgM production. 15B8 reduced IgM

production by about 30% in this assay (data not shown). Therefore, 15B8 can reduce T cell-mediated B cell immunoglobulin production.

In summary, 15B8 inhibits CD40L induced Bpcell/PBMC proliferation in human, chimp and marmoset, and inhibits T cell inducted antibody production by purified human B cells *in vitro*. These data demonstrate that 15B8 is an antagonistic anti-CD40 antibody in human B cells and PBMCs from chimps and marmosets *in vitro*.

EXAMPLE 16

15B8 IS AN AGONIST ANTI-MONKEY (CYNOMOLGUS, RHESUS AND BABOON) CD40 ANTIBODY *IN VITRO*.

FACS analysis demonstrates that 15B8 binds to CD40 expressed on the surface of B cells from peripheral blood of monkeys (rhesus, cynomologus and baboon). The effect of 15B8 on freshly isolated cynomolgus monkey PBMC was tested in the same proliferation assay described above for human and chimps (John E. Coligan et al., *Current Protocols in Immunology*, Vol. 13:12, John Wiley & Sons, Inc., 1991; Kwekkeboom J., *Immunology*, 1993 Jul, *79*(3):439-444). In contrast to human PBMC, 15B8 was found to stimulate cynomolgus monkey PBMC to proliferate *in vitro* as measured by ^{3}H methyl-thymidine incorporation (Table 13 below). At 1 μg/ml level, 15B8 stimulated the proliferation of the PBMCs by 6 - 129.7 folds compare to the huIgG2 control in the tewnety-two samples from seventeen monkeys tested (samples from five monekys were tested twice) (Table 13 below). At 5μg/ml level, the proliferation stimulated by 15B8 is 14 - 24 folds in four samples from to monkeys and about 1.25 or 1.85 fold in two samples from two monkeys (Table 13). This suggests that, at concentration level of 5μg/ml, 15B8 may be at the limit of over-saturating dose for its proliferation-stimulatory effect on PBMCs from cynomolgus monkey. Further FACS analysis of B cells for activation status by surface markers indicated that 15B8 induces CD69, CD86 and HLA-DR up-regulation on monkey B cells (Table 14). These data suggest that 15B8 is an agonist antibody to CD40 expressed on peripheral blood B cells from cynomolgus monkeys *in vitro*.

To confirm that this agonistic effect of 15B8 is not cynomolgus monkey specific, the same assays were performed using PBMCs from rhesus monkeys and

baboons. Similar results to that obtained from cells of cynomolgus monkeys were observed as shown in the Table 13 below. 15B8 stimulated proliferation of PBMCs from rhesus monkeys and baboons *in vitro* (Table 13 below). The agonist activity of 15B8 is shown using the PBMCs from five rhesus monkey and three baboons (Table 13).

Table 13

Proliferation of PBMCs from humans, cynomolgus and rhesus monkeys, and baboons stimulated at 15B8[1]

Species	cell source	Number of samples	dose (ug/ml)	hIgG2, base	CD40L, fold increase[3]	15B8, fold increase[2]
human	PBMC	5	5	1	9.36-91.60	0.49-2.28
		15	1	1	9.36-91.60	0.35-2.38
		12	0.2	1	9.36-91.60	0.41-3.74
Rhesus Monkey	PBMC	5	1	1	12.71-89.67	27.34-50.9
cyno monkey	PBMC	6	5	1	14.57-124.01	1.25-24.53
		22	1	1	5.15-167.73	6.13-129.74
		3	0.2	1	77.01-124.01	0.9-67.56
Baboon	PBMC	3	1	1	5.19-175.07	3.32-113.28

1, PBMCs were cultured in vitro in the presence of CD40L, 15B8 or huIgG2 control.
2. The proliferation results are reported as the ration of ^{3}H-thymidine incorporation for 15B8 to huIgG2 control.
Data from some samples are not in the table for the CPM induced by CD40L (positive control) <2000.
3. The fold-increase for CD40L shown in the table is the ratio of the CD40L cpm to the cpm of huIgG2 at 5μg/ml. CD40L transfected CHO cells were fixed with formaldehyde before the experiments.

Table 14

Effect of 15B8 on upregulation of B-cell activate markers *in vitro* by FACS analysis

Species	cell source	incubation time	Number of subject	CD54	CD69	HLA-DR	CD25	CD80	CD86
human	CD20 from PBMC	4 h-24 h	3	-	-	-	-	N/A	-
cyno monkey	CD20/19 from PBMC	4h-3 day	2	N/A	1/2 up	1/1 up (day 3)	-	-	1/1/up (day 3)

1, "-" means no up-regulation.
2, "N/A" means not measured or not successful.
3, Only cells from one cyno, analyzed by FACS on day 3.

EXAMPLE 17

15B8 IS AN AGONIST ANTI CD40 ANTIBODY *IN VIVO* IN CYNOMOLGUS MONKEYS..

15B8 can stimulate proliferation and up-regulation of cell surface activation markers in PBMCs from cynomolgus monkeys *in vitro*. To determine if 15B8 is an agonist anti-CD40 antibody in the monkeys *in vivo*, a study was performed to examine the biodistribution of 15B8 and the fate of affected peripheral B cells (i.e. extravastion, apoptosis, activation status, or complement lysis) [Biodistribution 15B8.72 Antibodies following Intravenous Administration to Non-Naïve Male and Female Cynomolgus Monkeys (SNBL.218.3, SNBL USA)].

Cynomolgus monkeys (1 female and 2 males) received a single intravenous administration of 3 mg/kg 15B8. The following parameters were monitored: clinical signs, food consumption, body weight, pharmacokinetics, serum complement (CH50), flow cytometry for B cells (including apoptotic B cells), T cells, and monocytes. B cell CD40 receptor saturation with 15B8 was also measured. Animals were necropsied 24 hours after receiving the single dose of 15B8, and standard organs were weighed. Pre-study surgical biopsies of spleen and axiliary lymph nodes were taken to serve as baseline controls. At necropsy, lymphoid and non-lymphoid tissues were sampled for histopathology and immunohistochemistry. Tissues were

immunostained with antibodies against CD3, CD40, CD20, CD27, and CD38 antigens. Preliminary results of the study are discussed below.

All animals survived to the scheduled necropsy and there were no effects on food consumption, body weight, CH50 levels nor on peripheral blood T cell or monocyte counts. There were no changes in organ weights. Microscopic examination of the spleen showed moderate diffuse follicular hyperplasia with necrosis and/or neutrophilic infiltrates in the germinal centers of all 15B8-treated animals. Examination of mesenteric and inguinal lymph nodes revealed mild follicular hyperplasia in 2/3 animals. No treatment related microscopic effects were seen in other tissues (liver, skin, brain, thyroid, lung, bone marrow, adrenal gland and kidney).

Immunostaining with CD20, CD27, CD40 and CD86 antibodies revealed increases in these markers in splenic and lymph node follicles, which correlated with the follicular hyperplasia seen in these same tissues. Increased staining of CD20 and CD40 were limited to the spleen and lymph node while there was some additional staining of hepatic tissue with CD27 and of hepatic Kupffer cells and inflammatory cells by CD86. CD86 staining was also increased in thymic medullary cells and adrenal interstitial leukocytes. There were no changes in the immunostaining of CD3 in 15B8-treated animals as compared to controls.

These findings indicate that a single dose of 3 mg/kg of 15B8 administered to cynomolgus monkey can cause proliferation of lymphoid follicles and/or redistribution of B cells from the peripheral blood in spleen and lymph nodes within a 24 hour period. Antibodies to CD20, CD27, CD40 and CD86 recognize antigens expressed on B cells and/or activated B cells, along with recognition of other cell types. Increased numbers of cells expressing these antigens were seen in the spleen and lymph nodes of treated animals, which suggests an increase in the number of activated CD20+ B cells. This study suggests that 15B8 is an agonist anti-CD40 antibody in cynomolgus monkey *in vivo*. The results obtained *in vivo* and *in vivo* are consistent in cynomolgus monkeys.

The results suggest that 15B8 is an agonistic anti-CD40 antibody in cynomolgus and rhesus monkeys and baboons, and an antagonistic antibody in humans, chimpanzees and marmosets.

15B8 is an anti-human CD40 specific monoclonal antibody with human IgG2 subtype and with cross-reactivity to CD40 from non-human primates only. Through extensive *in vitro* testing, 15B8 was shown to be an antagonistic anti-CD40 to the CD40 expressed on human B cells, PBMCs from human, chimp and marmoset. However, 15B8 was shown to have agonistic activity when bound to the CD40 expressed on PBMCs from monkeys (cynomolgus, rhesus and baboon) *in vitro*. This agonistic activity of 15B8 was confirmed *in vivo* in cynomolgus monkeys. When tested in primary culture of lymphoma cells from Rituxan-sensitive and resistant NHL patients, 15B8 has no agonist activity in the presence and absence of IL-4. 15B8 can also inhibit CD40L stimulated growth of the lymphoma cells from the similar group of patients under both conditions. 15B8 will have the potential to modify B cell malignancies, such as non-Hodgkin's lymphoma (NHL), where the CD40/CD40L pathway may play a role in the pathogenesis of the diseases. The results are summarized in the tables below.

Table 15

Assays Measuring Agonistic Activity

Assay	Methodology	Species Tested (+ or - Agonistic Activity)
Effect of 15B8 on B cell proliferation	Compared ^{3}H-thymidine incorporation of purified B cells from the peripheral blood in presence of 15B8 with incorporation in presence of CD40L or an agonistic antibody 626.1	• Human (-)
Effect of 15B8 on PBMC proliferation	Compared ^{3}H-thymidine incorporation of PBMCs in presence of 15B8 with incorporation in presence of CD40L or the isotype control	• Human (-) • Chimpanzee (-) • Cynomolgus monkey (+) •.Rhesus monkey (+) • Baboon (+) • Marmoset (-)
Effect of 15B8 on upregulation of B-cell activation markers	Measured upregulation in the expression of B-cell activation markers in PBMCs stimulated by 15B8 or it's isotype control using FACS analysis; compared effect of 15B8 with that of isotype control	• Human (-) •.Chimpanzee (-) •.Cynomolgus monkey (+) •.Rhesus monkey (+) •.Baboon (+) • Marmoset (-)
Effect on PBMC proliferation of 15B8 cross-linked to a secondary antibody fixed to a plastic surface	Compared ^{3}H-thymidine incorporation in presence of second Ab-crosslinked 15B8 with incorporation in presence of CD40L 15B8 alone or the isotype control	• Human (-) • Chimpanzee (-)
Effect of 15B8 on upregulation of FasL and apoptosis	Measured upregulation in the expression of FasL and apoptosis by FACS detection of B cells with positive staining of anti-FasL and Annexin V (marker for apoptosis) by the stimulation of CD40L, 15B8 and the isotype control.	• Human (-) • Chimps (-) • Cynomologus Monkey (-/+)

Table 16

Assays Measuring Antagonistic Activity

Assay	Methodology	Species Tested (+ or - Antagonistic Activity
Inhibition by 15B8 of CD40L-mediated B-cell proliferation	Stimulation of B-cell proliferation by CD40L-expressing CHO cells was measured by ^{3}H-thymidine incorporation. Compared ^{3}H-thymidine incorporation in presence of 15B8 with that in presence of isoitype control	• Human (+) • Marmoset (+) • Chimps (+)
Inhibition by 15138 of T-helper-cell-mediated B-cell antibody production	B cells were cultured with purified irradiated T cells activated with anti-CD3 antibody in the presence of 15B8. The level of B-cell IgM production was assessed by ELISA.	• Human (+)

The present invention has been described with reference to specific embodiments. However, this application is intended to cover those changes and substitutions which may be made by those skilled in the art without departing from the spirit and the scope of the appended claims.

CLAIMS

1. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human CD40-expressing cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal cell, the growth or differentiation of said normal cell is inhibited, wherein said antibody comprises a light chain complementarity determining region having a sequence selected from the group consisting of SEQ ID NO:19-30.

2. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human CD40-expressing cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal cell, the growth or differentiation of said normal cell is inhibited, wherein said antibody comprises a heavy chain complementarity determining region having a sequence selected from the group consisting of SEQ ID NO:19-30.

3. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human CD40-expressing cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal cell, the growth or differentiation of said normal cell is inhibited, wherein said antibody is secreted by a hybridoma selected from the group consisting of 15B8, 20C4, 12D9, 9F7, and 13E4.

4. The fragment of claim 1, 2, or 3 wherein said fragment is a member selected from the group consisting of an Fab′ fragment, an $F(ab)_2$ fragment, an Fab fragment, and an F_v fragment of said monoclonal antibody.

5. The monoclonal antibody of claim 1, 2 or 3 wherein said monoclonal antibody binds to said human CD40 antigen with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M, and at least 10^{-11} M.

6. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human B cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal B cell, the growth or differentiation of said normal B cell is inhibited, wherein said monoclonal antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 15, 20, 26, 32 and 38.

7. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human B cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal B cell, the growth or differentiation of said normal B cell is inhibited, wherein said monoclonal antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:13, 17, 21, 27, 33 and 39.

8. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human B cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal B cell, the growth or differentiation of said normal B cell is inhibited, wherein said monoclonal antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:12, 16, 22, 28, 34 and 40.

9. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human B cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal B cell, the growth or differentiation of said normal B cell is inhibited, wherein said monoclonal antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:14, 18, 23, 29, 35 and 41.

10. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human B cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal B cell, the growth or differentiation of said normal B cell is inhibited, wherein said monoclonal antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:24, 30, 36 and 42.

11. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human CD40-expressing cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal cell, the growth or differentiation of said normal cell is inhibited, wherein said monoclonal antibody is encoded by a nucleic acid comprising a polynucleotide selected from the group consisting of SEQ ID NO:1, 3, 4, 5, 6, 7, 8, 9, 10, and 43.

12. A nucleic acid comprising a polynucleotide that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:20-24, 26-30, 32-36, and 38-42, wherein said amino acid sequence contains at least one of the light

chain and heavy chain complementarity determining regions of an antibody capable of specifically binding to CD40 on normal human B cells.

13. A human monoclonal antibody or fragment thereof that is capable of specifically binding to a human CD40 antigen expressed on the surface of a normal human CD40-expressing cell, said monoclonal antibody or fragment being free of significant agonistic activity, whereby, when said monoclonal antibody or fragment binds to the CD40 antigen expressed on the surface of said normal cell, the growth or differentiation of said normal cell is inhibited, wherein said antibody or fragment thereof is produced by a hybridoma selected from the group consisting of 15B8, 20C4, 13E4, 12D9, and 9F7.

14. The monoclonal antibody of any one of claims 7-11 wherein said monoclonal antibody binds to said human CD40 antigen with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M, and at least 10^{-11} M.

15. A method for inhibiting growth or differentiation of a normal human B cell, said method comprising contacting said B cell with an effective amount of a human anti-CD40 monoclonal antibody, said antibody being free of significant agonistic activity, whereby when said antibody binds to said CD40 antigen on said B cell, the growth or differentiation of said B cell is inhibited, wherein said monoclonal antibody binds to said human B cell with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M, and at least 10^{-11} M.

16. A method for inhibiting growth or differentiation of a normal human B cell, said method comprising contacting said B cell with an effective amount of a human anti-CD40 monoclonal antibody, said antibody being free of significant agonistic activity, whereby when said antibody binds to said CD40 antigen on said B cell, the growth or differentiation of said B cell is inhibited, wherein said human

monoclonal antibody is selected from the group consisting of 15B8, 20C4, 13E4, 12D9, and 9F7.

17. The method of claim 15, wherein said monoclonal antibody is an Fab′ fragment, an $F(ab)_2$ fragment, an Fab fragment, or an F_v fragment of said monoclonal antibody.

18. The method of claim 16, wherein said monoclonal antibody is an Fab′ fragment, an $F(ab)_2$ fragment, an Fab fragment, or an F_v fragment of said monoclonal antibody.

19. A method for inhibiting proliferation of a normal human B cell, wherein said proliferation is augmented by the interaction of a CD40 ligand with a CD40 antigen expressed on the surface of a B cell, said method comprising contacting said B cell with an effective amount of a human anti-CD40 monoclonal antibody, said antibody being free of significant agonostic activity, whereby when said antibody binds to said CD40 antigen on said B cell, the proliferation of said B cell is inhibited, wherein said monoclonal antibody binds to said human B cell with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M, and at least 10^{-11} M.

20. A method for inhibiting proliferation of a normal human B cell, wherein said proliferation is augmented by the interaction of a CD40 ligand with a CD40 antigen expressed on the surface of a B cell, said method comprising contacting said B cell with an effective amount of a human anti-CD40 monoclonal antibody, said antibody being free of significant agonostic activity, whereby when said antibody binds to said CD40 antigen on said B cell, the proliferation of said B cell is inhibited, wherein said monoclonal antibody binds to said human B cell with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M, and at least 10^{-11} M wherein said human monoclonal antibody is selected from the group consisting of 15B8, 20C4, 13E4, 12D9, and 9F7.

21. A method for inhibiting antibody production by B cells in a human patient, said method comprising administering to a human patient an effective amount of a human anti-CD40 monoclonal antibody, said antibody being free of significant agonostic activity, whereby when said antibody binds to said CD40 antigen on said B cells, antibody production by said B cells is inhibited, wherein said monoclonal antibody binds to said human B cell with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M and at least 10^{-11} M.

22. A method for inhibiting antibody production by B cells in a human patient, said method comprising administering to a human patient an effective amount of a human anti-CD40 monoclonal antibody, said antibody being free of significant agonostic activity, whereby when said antibody binds to said CD40 antigen on said B cells, antibody production by said B cells is inhibited, wherein said monoclonal antibody binds to said human B cell with an affinity (K_D) selected from the group consisting of at least 10^{-5} M, at least 10^{-7} M, at least 10^{-9} M and at least 10^{-11} M, wherein said human monoclonal antibody is selected from the group consisting of 15B8, 20C4, 13E4, 12D9, and 9F7.

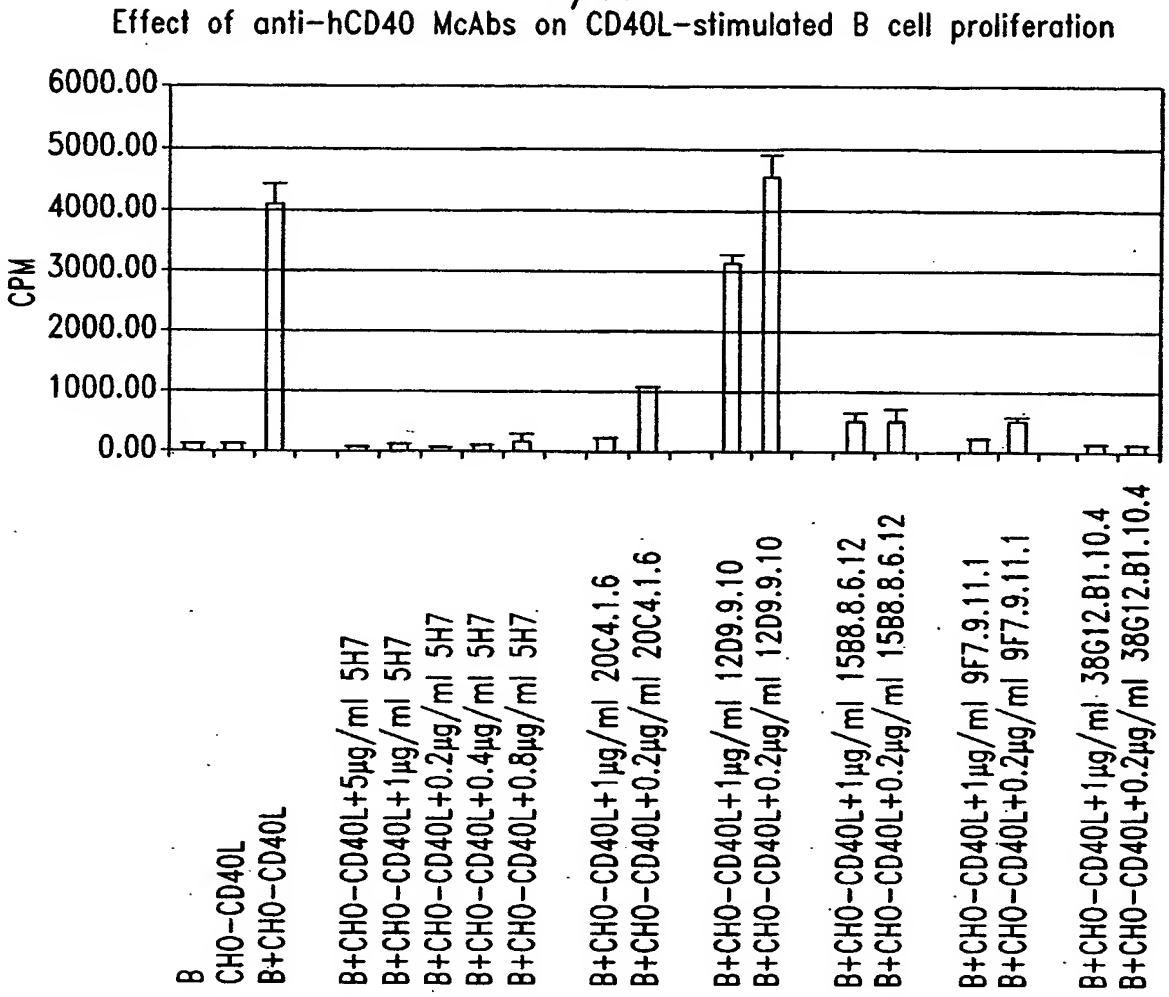

Fig. 1

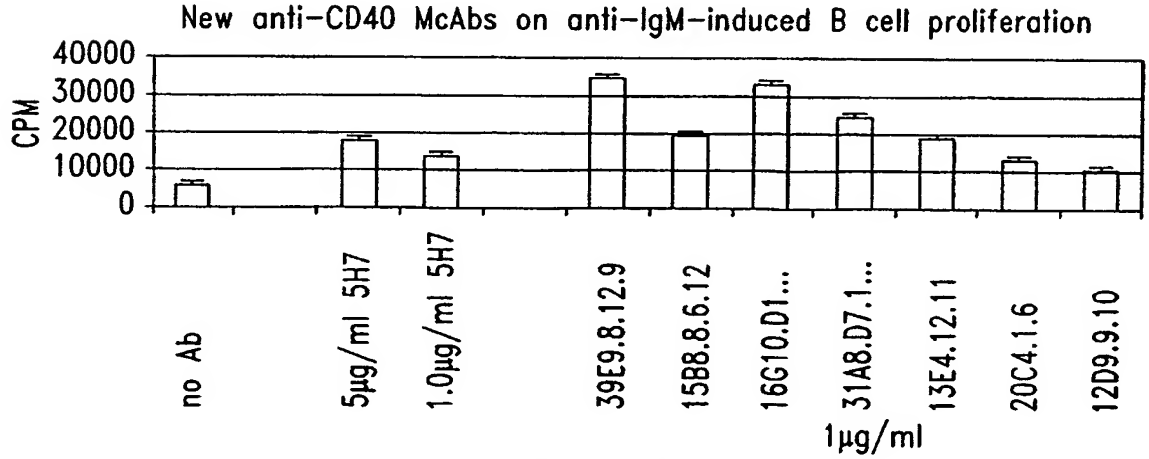

Fig. 3

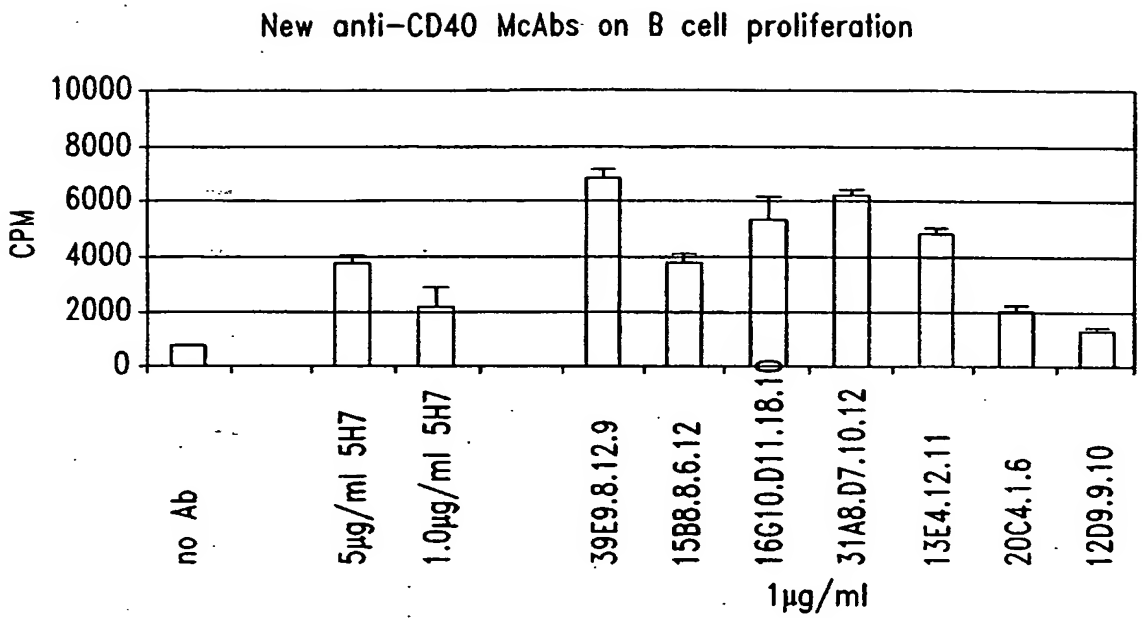
New anti-CD40 McAbs on B cell proliferation
CPM
10000
8000
6000
4000
2000
0
no Ab
5µg/ml 5H7
1.0µg/ml 5H7
39E9.8.12.9
15B8.8.6.12
16G10.D11.18.10
31A8.D7.10.12
13E4.12.11
20C4.1.6
12D9.9.10
1µg/ml

Fig. 2

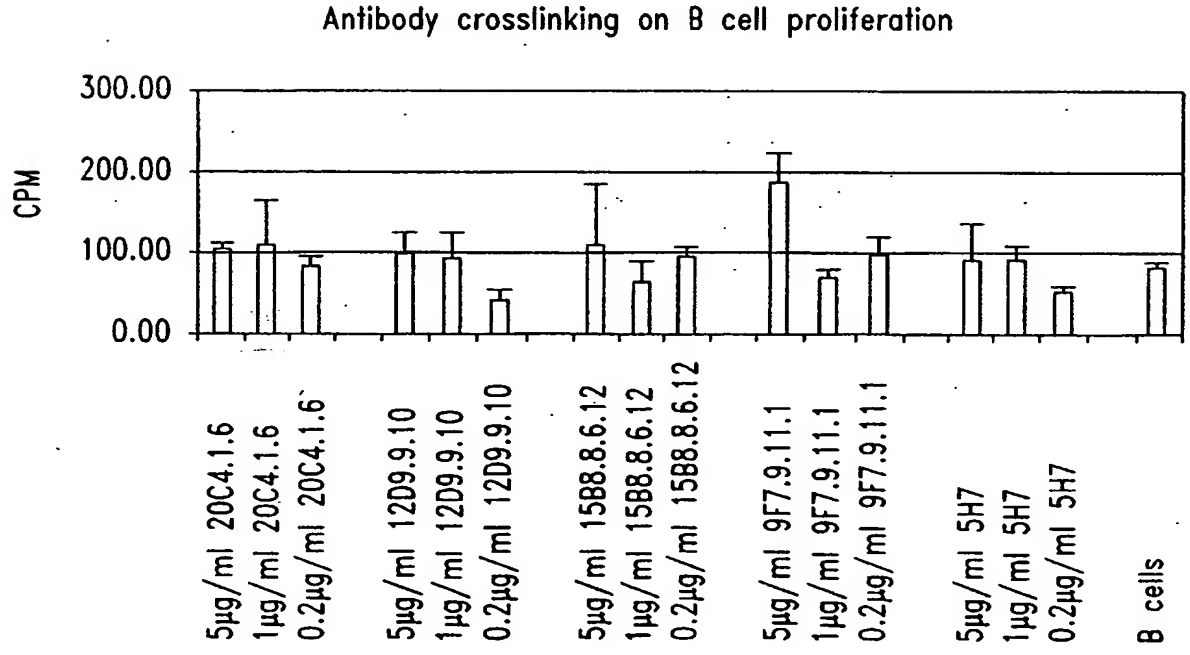
Antibody crosslinking on B cell proliferation
CPM
300.00
200.00
100.00
0.00
5µg/ml 20C4.1.6
1µg/ml 20C4.1.6
0.2µg/ml 20C4.1.6
5µg/ml 12D9.9.10
1µg/ml 12D9.9.10
0.2µg/ml 12D9.9.10
5µg/ml 15B8.8.6.12
1µg/ml 15B8.8.6.12
0.2µg/ml 15B8.8.6.12
5µg/ml 9F7.9.11.1
1µg/ml 9F7.9.11.1
0.2µg/ml 9F7.9.11.1
5µg/ml 5H7
1µg/ml 5H7
0.2µg/ml 5H7
B cells

Fig. 4

Sequence 2. Light chain deduced amino acid sequence of selected hybridomas compared with 5H7

```
Frm1                          CDR1          Frm2              CDR2
5H7  DIVITQAPLSLPVSLGDQASISCRSSQSLVNSNGNTYLHWYLQKPGQSPKLLIYKVSNRFS
9F7  -VLM--T-------------------TI-H---H---E-------------S-------
15B8 ---M--S----S-AP-QP-----K-----LE-V-Q---Y--------P-Q----AVFK---
12D9 E--L--S-GT-SL-P-ER-TL---A---    VSYSYLA--Q-----A-R----GA-S-AT
20C4 E--L--S-GT-SL-P-EG-TL---A---    VSYSYLA--Q-----A-R----GA-S-AT
13E4 --QL--S-S--SA-V--RVT-T--A--G    IRN DLG--Q-----A-RR---AA-SLQ-

Frm3                                  CDR3     Frm4
5H7  GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLELK
9F7  ----------------------P----I-Y-F-GS---F---P---VYI-
15B8 -------------------------V---Y-M--MQL-L-------V-I-
12D9 -I--------------T---L-P--FA--Y-Q-YGSSFR---Q---Q-I-
20C4 -I--------------T---L-P--FA--Y-H-YGNSFR---Q---V-I-
13E4 ---S-----R---E---T--SLQP--PAT-Y-L-HNSS-CS--Q-----I-
```

Fig. 5

Heavy Chain

	Frm1	CDR1	Frm2	CDR2
5H7	QVQLQESGPGLVAPSQSLSITCTVSGFSLS	RYSVY	WVRQPPGKGLEWLG	MMWGGGSTDYNSALKS
9F7	--------G-V-Q-GR--RLS-AA---TF-	S-GMH	----A-------VA	VISYD--IK-YADSVKG
12D9	--------G-V-Q-GR--RLS-AA---TF-	S-GMD	----A-------VA	VLSYD--NK-YADSVKG
13E4	-----Q--AEVKK-GA-VKVS-KA--YTFT	D-YMH	----A--Q----M-	WINPNSGGTNYAQKFQG
15B8	--------G-V-Q-GR--RLS-AA---TFN	NFGIH	----A-------VA	VISYD--DK-YADSVKG
20C4	--------G-V-Q-GR--RLS-AA---TF-	S-GMD	----A-------VA	VLSYA--NK-YADSVKG

	Frm3	CDR3	Frm4
5H7	RLSISKDTSKSQVFLKMNSLQTDDTAMYYCVR	TDG DY	WGQGTSVTVSS
9F7	-FT--R-N--NTLY-Q----RAE---V---A-	DH-SNPL --	-----L-----
12D9	-FT--R-N--NTLY-QL---RAE---V---A-	DTVRGF --	----IL-----
13E4	-VTMTR---I-IAYMELDR-RS----V---A-	DEILAADGIYYFYGL-V	-----T-----
15B8	-FT--R-N--NTLN-Q----RAE---V---A-	DRRYYYHYYGM -V	-----M-----
20C4	-FT--R-N--NTLY-Q----RPE---V---A-	DTVRGF -Y	----IL-----

Light Chain

	Frm1	CDR1	Frm2	CDR2
5H7	DIVITQAPLSLPVSLGDQASISC	RSSQSLVNSNGNTYLH	WYLQKPGQSPKLLIY	KVSNRFS
9f7	-VLM--T----------	----TI-H---H---E	----------S	-------
12D9	E--L--S-GT-SL-P-ER-TL--	-A--- VSYSYLA	--Q-----A-R----	GA-S-AT
13E4	--QL--S-S--SA-V--RVT-T-	-A--G IRN DLG	--Q-----A-RR---	AA-SLQ-
15B8	---M--S----S-AP-QP-----	K-----LE-Y-E---Y	--------P-Q----	AVFK---
20C4	E--L--S-GT-SL-P-EG-TL--	-A--- VSYSYLA	--Q-----A-R----	GA-S-AT

	Frm3	CDR3	Frm4
5H7	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	SQSTHVPWT	FGGGTKLELK
9F7	----------P----I-Y-	F-GS---F-	--P---VYI-
12D9	-I----------T---L-P--FA--Y-	Q-YGSSFR-	--Q---Q-I-
13E4	---S-----R---E---T--SLQP--FAT-Y-	L-HNSS-CS	--Q-----I-
15B8	----------V---Y-	M--MQL-L-	------V-I-
20C4	-I----------T---L-P--FA--Y-	H-YGNSFR-	--Q---V-I-

Fig. 6

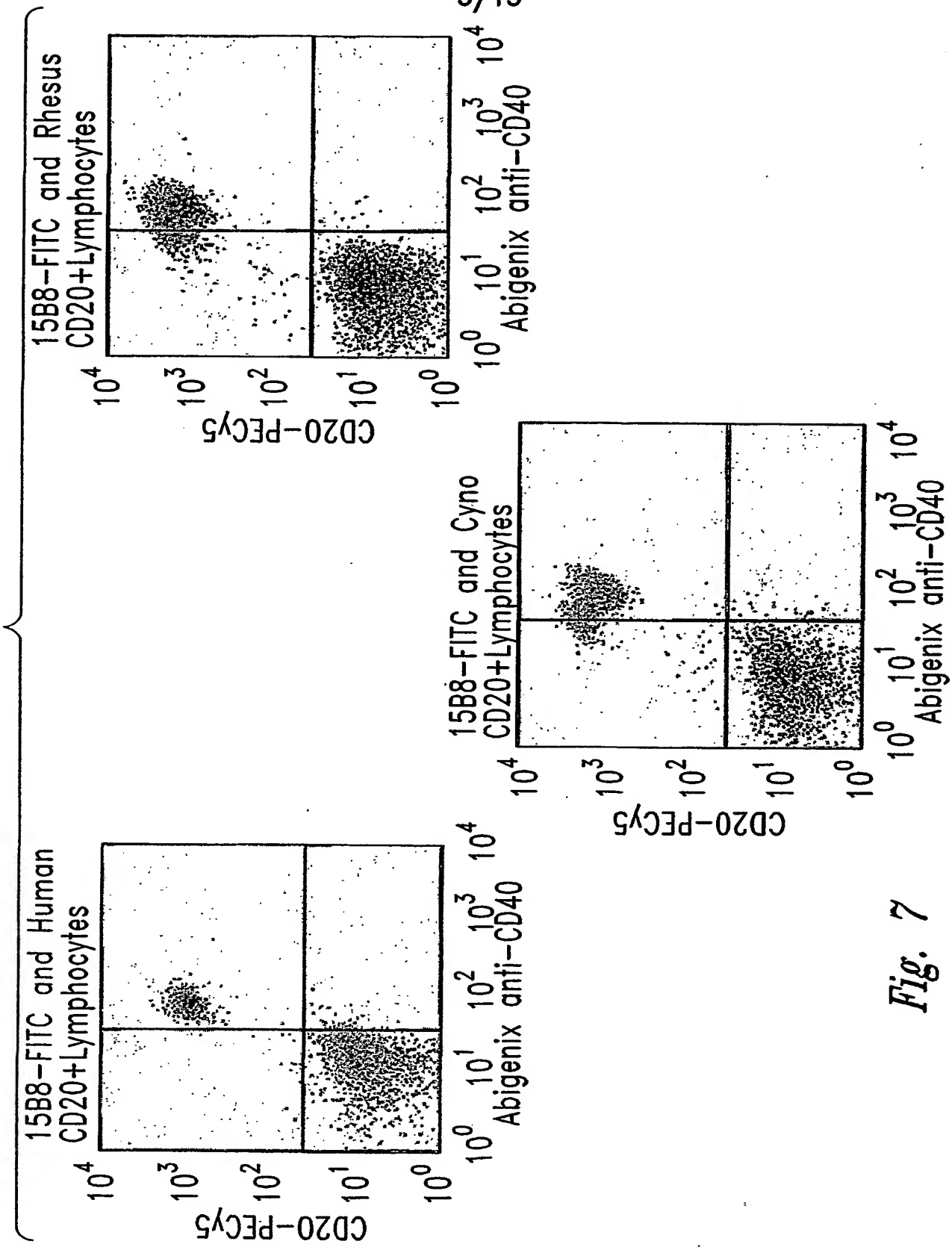

Fig. 7

12D9vk

```
+2              E  I  V  L  T  Q  S  P  G  T  L  S 1
              GAAATTG TGTTGACGCA GTCTCCAGGC ACCCTGTCTT
              GGCTTTAAC ACAACTGCGT CAGAGGTCCG TGGGACAGAA

+2 L  S  P  G  E  R  A  T  L  S  C  R  A  S  Q  S  V
51 TGTCTCCAGG GGAAAGAGCC ACCCTCTCCT GCAGGGCCAG TCAGAGTGTT
   ACAGAGGTCC CCTTTCTCGG TGGGAGAGGA CGTCCCGGTC AGTCTCACAA

+2 S  Y  S  Y  L  A  W  Y  Q  Q  K  P  G  Q  A  P  R
101 AGCTACAGCT ACTTAGCCTG GTACCAGCAG AAACCTGGCC AGGCTCCCAG
    TCGATGTCGA TGAATCGGAC CATGGTCGTC TTTGGACCGG TCCGAGGGTC

+2  L  L  I  Y  G  A  S  S  R  A  T  G  I  P  D  R
151 ACTCCTCATC TATGGTGCAT CCAGCAGGGC CACTGGCATC CCAGACAGGT
    TGAGGAGTAG ATACCACGTA GGTCGTCCCG GTGACCGTAG GGTCTGTCCA

+2 F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  R  L
201 TCAGTGGCAG TGGGTCTGGA ACAGACTTCA CTCTCACCAT CAGCAGACTG
    AGTCACCGTC ACCCAGACCT TGTCTGAAGT GAGAGTGGTA GTCGTCTGAC

+2 E  P  E  D  F  A  V  Y  Y  C  Q  Q  Y  G  S  S  F
251 GAGCCTGAGG ATTTTGCAGT GTATTACTGT CAGCAGTATG GTAGCTCATT
    CTCGGACTCC TAAAACGTCA CATAATGACA GTCGTCATAC CATCGAGTAA

+2 R  T  F  G  Q  G  T  K  Q  E  I  K
301 TCGGACGTTC GGCCAAGGGA CCAAGCAGGA GATCAAA
    AGCCTGCAAG CCGGTTCCCT GGTTCGTCCT CTAGTTT
```

Fig. 8

12d9Const

```
GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCCGAG     60
AGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG    120
TGGAACTCAG GCGCTCTGAC CAGCGGCGTG CACACCTTCC CAGCTGTCCT ACAGTCCTCA    180
GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAACTTCGG CACCCAGACC    240
TACACCTGCA ACGTAGATCA CAAGCCCAGC AACACCAAGG TGGACAAGAC AGTTGAGCGC    300
AAATGTTGTG TCGAGTGCCC ACCGTGCCCA GCACCACCTG TGGCAGGACC GTCAGTCTTC    360
CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACGTGC    420
GTGGTGGTGG ACGTGAGCCA CGAAGACCCC GAGGTCCAGT TCAACTGGTA CGTGGACGGC    480
GTGGAGGTGC ATAATGCCAA GACAAAGCCA CGGGAGGAGC AGTTCAACAG CACGTTCCGT    540
GTGGTCAGCG TCCTCACCGT TGTGCACCAG GACTGGCTGA ACGGCAAGGA GTACAAGTGC    600
AAGGTCTCCA ACAAAGGCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AACCAAAGGG    660
CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT GACCAAGAAC    720
CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTACCCCA GCGACATCGC CGTGGAGTGG    780
GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACAC CTCCCATGCT GGACTCCGAC    840
GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC    900
GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC    960
TCCCTGTCTC CGGGTAAA                                                  978
```

```
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS     60
GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVER KCCVECPPCP APPVAGPSVF    120
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR    180
VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN    240
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN    300
VFSCSVMHEA LHNHYTQKSL SLSPGK                                         326
```

Fig. 9

20C4vk.1

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20C4vh1

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Fig. 10

9F7vk.1

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TCTGGGGTCC CAGACAGGTT CAGTGGCAGT GGATCAGGGA CAGATTTCAC ACTCAAGATC  240
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9F7vh1

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CCAGGCAAGG GGCTGGAGTG GGTGGCAGTT ATATCATATG ATGGAAGTAT TAAATACTAT  180
GCTGACTCCG TGAAGGGCCG ATTCACCATC TCCAGAGACA ATTCCAAGAA CACGCTGTAT  240
CTGCAAATGA ACAGCCTGAG AGCTGAGGAC ACGGCTGTGT ACTACTGTGC GAGAGATCAT  300
GGGAGTAACC CCCTTGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA         354
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Fig. 11

15B8vk.3

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CTCACTTTCG GCGGAGGGAC CAAGGTGGAG ATCAAA 336

15B8vh1

CAGGTGCAGC TGCAGGAGTC TGGGGGAGGC GTGGTCCAGC CTGGGAGGTC CCTGAGACTC 60
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TCCTCA 366

Fig. 12

13E4vh1

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12D9vh1

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Fig. 13

9F7VH1
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Fig. 14

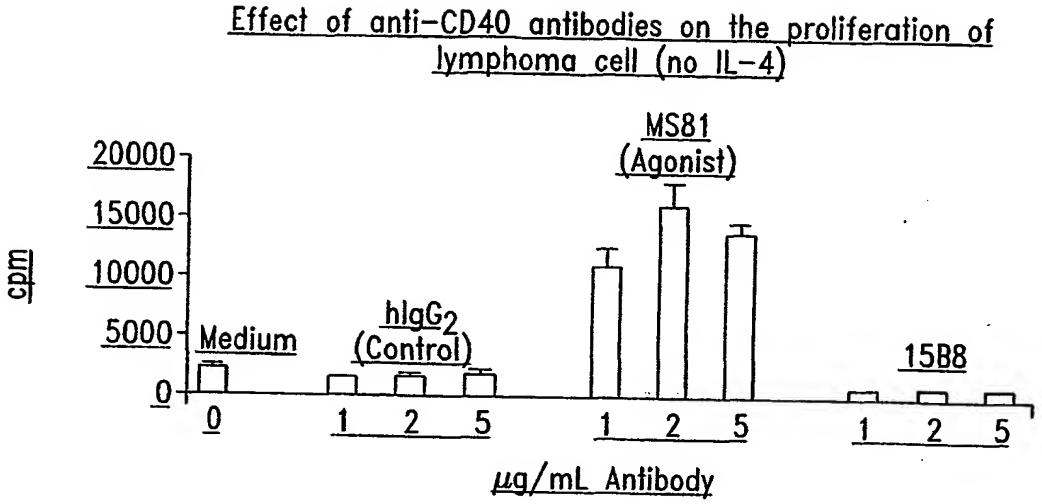
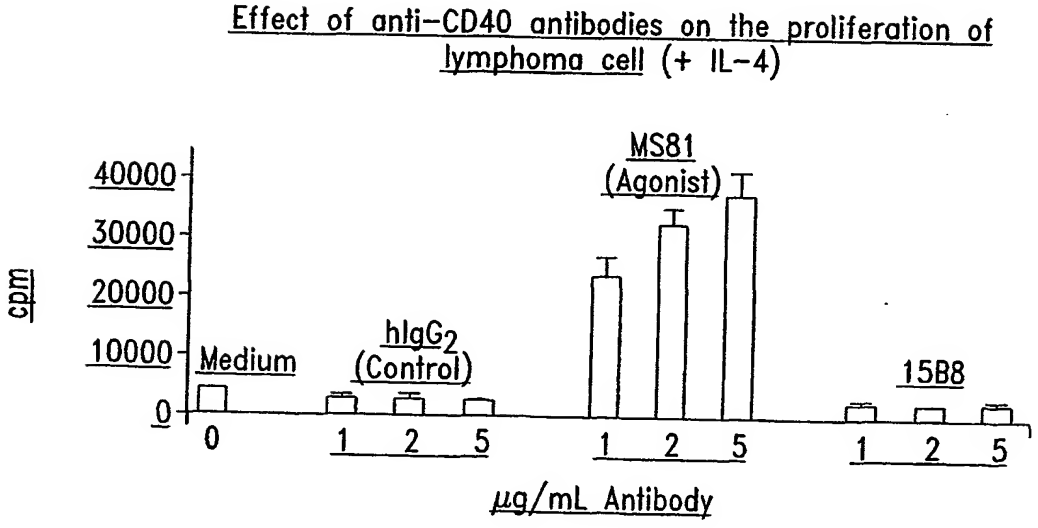

Effect of anti-CD40 antibodies on the proliferation of lymphoma cell (no IL-4)
cpm
20000
15000
10000
5000
0
Medium
hIgG2 (Control)
MS81 (Agonist)
15B8
0
1 2 5
1 2 5
1 2 5
µg/mL Antibody

Fig. 15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cpm
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30000
20000
10000
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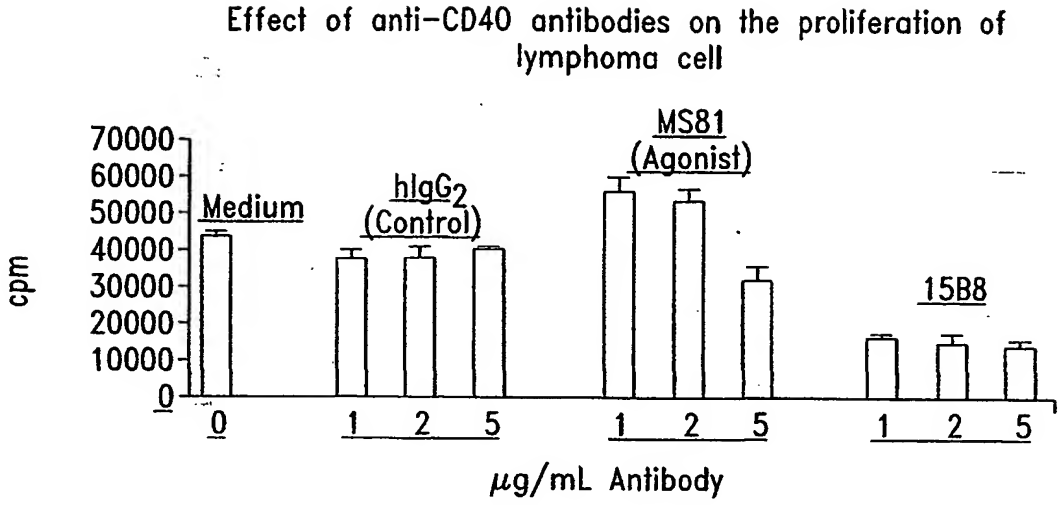
Medium
hIgG2 (Control)
MS81 (Agonist)
15B8
0
1 2 5
1 2 5
1 2 5
µg/mL Antibody

Fig. 16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cpm
70000
60000
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40000
30000
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10000
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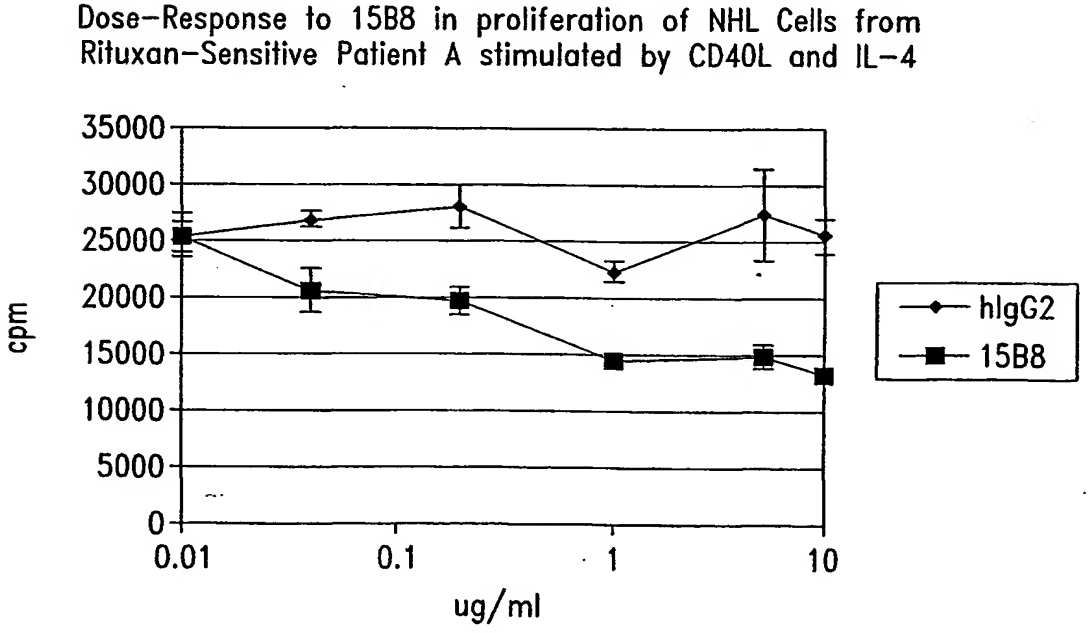
Medium
hIgG2 (Control)
MS81 (Agonist)
15B8
0
1 2 5
1 2 5
1 2 5
μg/mL Antibody

Fig. 17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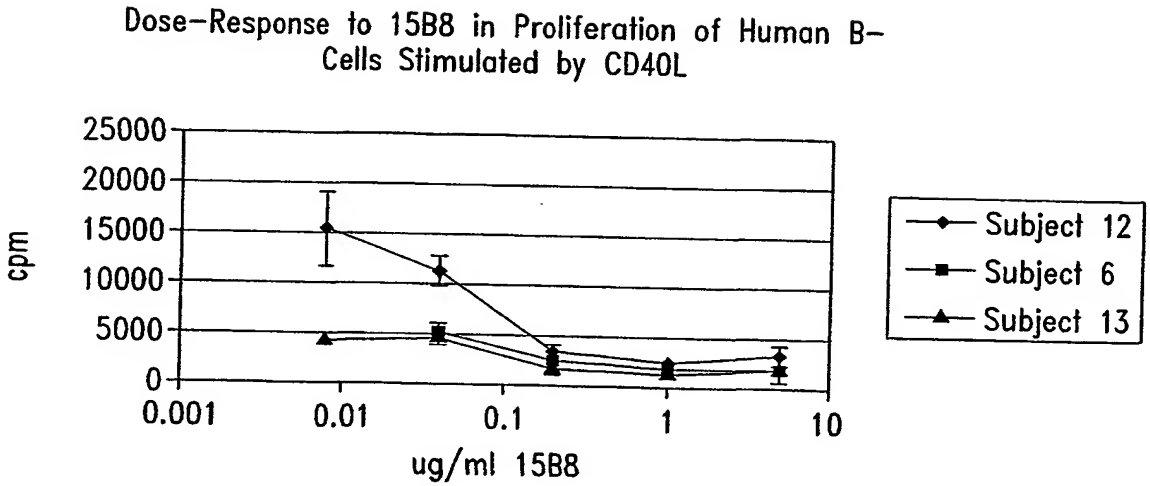
0.01
0.1
1
10
ug/ml
hIgG2
15B8

Fig. 18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1
10
ug/ml 15B8
Subject 12
Subject 6
Subject 13

Fig. 19

SEQUENCE LISTING

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Yoshihara, Corrine
Donnelly, John J.

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Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
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Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140
Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
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Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
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Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Asn
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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        35                  40                  45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Phe
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Arg Thr Phe Gly Gln Gly Thr Lys Gln
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<210> 17
<211> 112
<212> PRT
<213> Homo sapiens

<400> 17

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Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Glu Ser
            20                  25                  30
Tyr Gly Glu Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Pro
        35                  40                  45
Pro Gln Leu Leu Ile Tyr Ala Val Phe Lys Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
                85                  90                  95
Met Gln Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
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<210> 18
<211> 108
<212> PRT
<213> Homo sapiens

<400> 18

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Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1               5                  10                  15
Glu Gly Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Tyr Ser
            20                  25                  30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys His Gln Tyr Gly Asn Ser Phe
                85                  90                  95
Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

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(74) Agents: **ALEXANDER, Lisa**; Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916 et al. (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
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(88) Date of publication of the international search report:
6 February 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HUMAN ANTI-CD40 ANTIBODIES

(57) Abstract: Human antibodies capable of binding CD40 are disclosed, wherein the antibodies act as antagonists of CD40-directed activities of B cells, but have no or minimal ability to induce B cell proliferation. The antibodies are useful for treating diseases mediated by CD40-expressing cells, such as those diseases characterized by B cell activation, as well as cancer of B-cell lineage, including Non-Hodgkin's Lymphoma.

WO 02/028904 A3

INTERNATIONAL SEARCH REPORT

Inte al Application No
PCT/US 01/30857

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/28 C12N15/13 A61K39/395 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. KARLSSON ET AL.: "Selection of human single chain antibodies against CD40." IMMUNOLOGY LETTERS, vol. 73, no. 2-3, September 2000 (2000-09), page 161 XP001073504 Amsterdam, The Netherlands abstract 358 ---	1-22
A	EP 0 945 465 A (CHIRON CORPORATION) 29 September 1999 (1999-09-29) examples claims ---	1-22
A	WO 96 34096 A (CELL GENESYS INC.) 31 October 1996 (1996-10-31) claims 1,14,17,18,24,25 --- -/--	1-22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2002	07/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 83755 A (GEMINI SCIENCE, INC.) 8 November 2001 (2001-11-08) example 3 claims	15, 17-19,21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/30857

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 15-20 (all partially, as far as an in vivo method is concerned) and claims 21 and 22 (both completely) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/US 01/30857

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 945465	A	29-09-1999	US	5397703 A	14-03-1995
			US	5869050 A	09-02-1999
			US	5677165 A	14-10-1997
			EP	0945465 A1	29-09-1999
			CA	2125472 A1	20-01-1994
			EP	0651797 A1	10-05-1995
			JP	7509359 T	19-10-1995
			WO	9401547 A2	20-01-1994
			US	5747034 A	05-05-1998
			US	6056959 A	02-05-2000
			US	6004552 A	21-12-1999
			US	6315998 B1	13-11-2001
			US	5874082 A	23-02-1999
			US	2002106371 A1	08-08-2002
WO 9634096	A	31-10-1996	CA	2219486 A1	31-10-1996
			WO	9634096 A1	31-10-1996
			AU	2466895 A	18-11-1996
			EP	0823941 A1	18-02-1998
			JP	11505107 T	18-05-1999
WO 0183755	A	08-11-2001	AU	5921501 A	12-11-2001
			WO	0183755 A2	08-11-2001